317

UNIVERSITY OF MICHIGAN
JUN 25 1956
SCIENCE LIBRARY

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ARNE MÜNTZING

BAND 42: 1—2 1956

BERLINGSKA BOKTRYCKERIET, LUND

HEREDITAS

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (42) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Associate Editor. Price *Forty (40) Swedish Kronor per volume.* Postage free.

Back volumes. — The price of Vols. 1—12 and 19—20 is 15 Sw. Kr. each, of Vols. 26—33 and 37, 25 Sw. Kr. each, and of Vols. 39—41 35 Sw. Kr. each. Postage free.

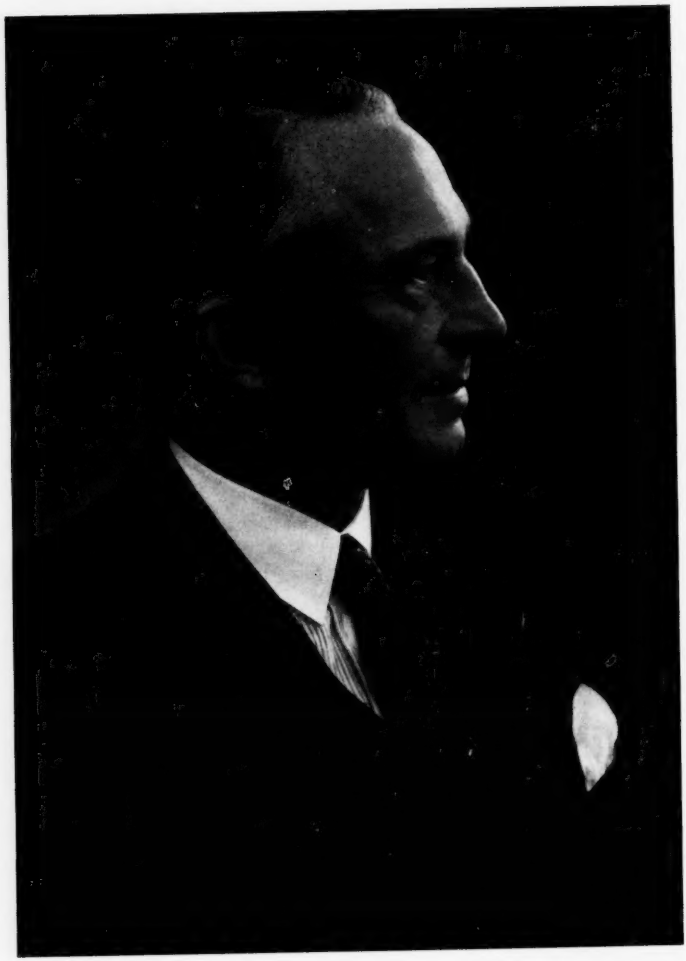
(Vols. 13—18, 21—25, 34—36, 38 and Suppl. Vol. 1949 are entirely out of print.)

ARNE MÜNTZING
Editor
Institute of Genetics,
LUND, SWEDEN

PETER BERNSTRÖM
Associate Editor
Hilleshög Sugar Beet Breeding Institute
LANDSKRONA, SWEDEN

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *J. RASMUSSON*
PROFESSOR, FIL. DR. *ÅKE GUSTAFSSON*
PROFESSOR, FIL. DR. *GERT BONNIER*
PROFESSOR, MED. DR. *ERIK ESSEN-MÖLLER*



Professor

Øjvind Winge

who celebrates his 70th anniversary on the 19th of May this year, started his scientific production as a mycologist and published his first papers on fungi as early as 1907. Although much of his time was later devoted to cytogenetic problems in animals and higher plants, he finally returned to the fungi, on which his main interest has been centred during the last twenty years. As Director of the Physiological Department of the Carlsberg Laboratory, Copenhagen, his research work has been focused on yeast, a material in which Winge and his collaborators have obtained results of fundamental importance not only for brewery work but for genetics in general. Winge clarified the occurrence of haplophase and diplophase in the yeast species, demonstrated that hybridization can be performed and that segregation occurs in the offspring of heterozygotes. Other papers on yeast deal with hybrid vigour, balanced heterozygosity, mutations and cytoplasmatic effects. Gene-analytical studies revealed the occurrence of polymeric, dominant gense for maltose, raffinose and sucrose fermentation. In a recent paper the causes of deviation from 2:2 segregations in the tetrads of monohybrid yeasts are discussed.

As a young cytologist Winge was attracted by the phenomenon of polyploidy, and in 1917 he formulated the well-known theory, soon to be verified, that polyploid series may arise from hybridization and summation of the chromosome sets of the parent species.

One of his most important fields of research has been sex chromosomes and sex-linked inheritance in various plant species (especially Melandrium and Humulus) and in the fish Lebistes reticulatus. Besides identification and localization of numerous colour genes the Lebistes work led to the

general conclusion that sex-determining genes are present in all the chromosomes of the species and that sex chromosomes may be changed to autosomes and vice versa.

In a paper of 1924 Winge revealed that the so-called speltoid mutants in wheat are associated with meiotic disturbances and elimination of certain chromosomes. A cytological key to a puzzling genetic phenomenon was also given in the case of Erophila verna. Winge showed that the great morphological variation in this collective species is associated with a chromosomal polymorphism, and that new microspecies, some of them amphidiploid, may be formed by hybridization.

Winge was also the first cytogeneticist to deal with tumour tissues as genetically heterogeneous cell populations, and to visualize the origin and growth of cancer as the result of selective processes among the constituents of this population. These ideas were based on careful and extensive chromosome studies in plant as well as animal tumours. His view of the neoplastic tissue as a genetic mosaic, in which the most viable cell lineages are selected to propagate the tumour, stands out as the first, and for a long period of time, the only attempt to extend the realms of cytogenetics from the level of organisms to the level of tissues. This work furnishes a fine example of his unusual ability of concentrating on a problem and of reaching essential results. Another characteristic feature is his versatility, which has enabled him to make important contributions in such diverse fields as human genetics, non-mendelian inheritance in plants, linkage in Pisum and the inheritance of colours in horses. Other papers deal with the practical applications of genetics in poultry, dogs, cattle, forest trees and Man. His vast knowledge of the whole field of genetics and cytology is well reflected in a widely used textbook of genetics (3rd edition 1945).

Through the medium of this journal Nordic geneticists wish to pay tribute to Øjvind Winge for his outstanding work over a period of half a century, at the same time expressing their best wishes for the years to come.

THE CHROMOSOME NUMBER OF MAN

By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

WHILE staying last summer at the Sloan-Kettering Institute, New York, one of us tried out some modifications of HSU's technique (1952) on various human tissue cultures carried in serial *in vitro* cultivation at that institute. The results were promising inasmuch as some fairly satisfactory chromosome analyses were obtained in cultures both of tissues of normal origin and of tumours (LEVAN, 1956).

Later on both authors, working in cooperation at Lund, have tried still further to improve the technique. We had access to tissue cultures of human embryonic lung fibroblasts, grown in bovine amniotic fluid; these were very kindly supplied to us by Dr. RUNE GRUBB of the Virus Laboratory, Institute of Bacteriology, Lund. All cultures were primary explants taken from human embryos obtained after legal abortions. The embryos were 10—25 cm in length. The chromosomes were studied a few days after the *in vitro* explantation had been made.

In our opinion the hypotonic pre-treatment introduced by HSU, although a very significant improvement especially for spreading the chromosomes, has a tendency to make the chromosome outlines somewhat blurred and vague. We consequently tried to abbreviate the hypotonic treatment to a minimum, hoping to induce the scattering of the chromosomes without unfavourable effects on the chromosome surface. Pre-treatment with hypotonic solution for only one or two minutes gave good results. In addition, we gave a colchicine dose to the culture medium 12—20 hours before fixation, making the medium 50×10^{-9} mol/l for the drug. The colchicine effected a considerable accumulation of mitoses and a varying degree of chromosome contraction. Fixation followed in 60 % acetic acid, twice exchanged in order to wash out the salts left from the culture medium and from the hypotonic solution that would otherwise have caused precipitation with the orcein. Ordinary squash preparations were made in 1 % acetic orcein. For chromosome counts the squashing was made very mild in order to keep the chromosomes in the metaphase groups. For idiogram studies a more thorough squashing was preferable. In many cases single cells were squashed

under the microscope by a slight pressure of a needle. In such cases it was directly observed that no chromosomes escaped.

THE CHROMOSOME NUMBER

With the technique used exact counts could be made in a great number of cells. Figs. 1 *a* and *b* represent typical samples of the appearance of the chromosomes at early metaphase (*a*) and full metaphase (*b*), showing the ease with which the counting could be made. In Table 1 the numbers of counts made from the four embryos studied are recorded.

TABLE 1. *Number of exact chromosome counts made.*

Embryo No.	Number of cultures	Number of counts
1	5	15
2	10	98
3	3	119
4	4	29
Total	22	261

We were surprised to find that the chromosome number 46 predominated in the tissue cultures from all four embryos, only single cases deviating from this number. Lower numbers were frequent, of course, but always in cells that seemed damaged. These were consequently disregarded just as the solitary chromosomes and the groups with but a few chromosomes, which were frequent. In some doubtful cases the numbers 47 and 48 were counted (in four cases not included in the table). This may be due to one or two solitary chromosomes having been pressed into a 46-chromosome plate at the squashing. It is also possible that deviating numbers may originate through non-disjunction, thus representing a real chromosome number variation in the living tissue. This kind of variation will probably increase as a consequence of the change in environment for the tissue involved in the *in vitro* explantation. HSU (1952) reports a certain degree of such variation in his primary cultures. LEVAN (1956), studying long-carried serial subcultures, found hypotriploid stemline numbers in two of them, and a near-diploid number in a third culture. In this culture one cell with 48 chromosomes was analysed. Naturally, at that time, this was thought to represent the normal diploid number.

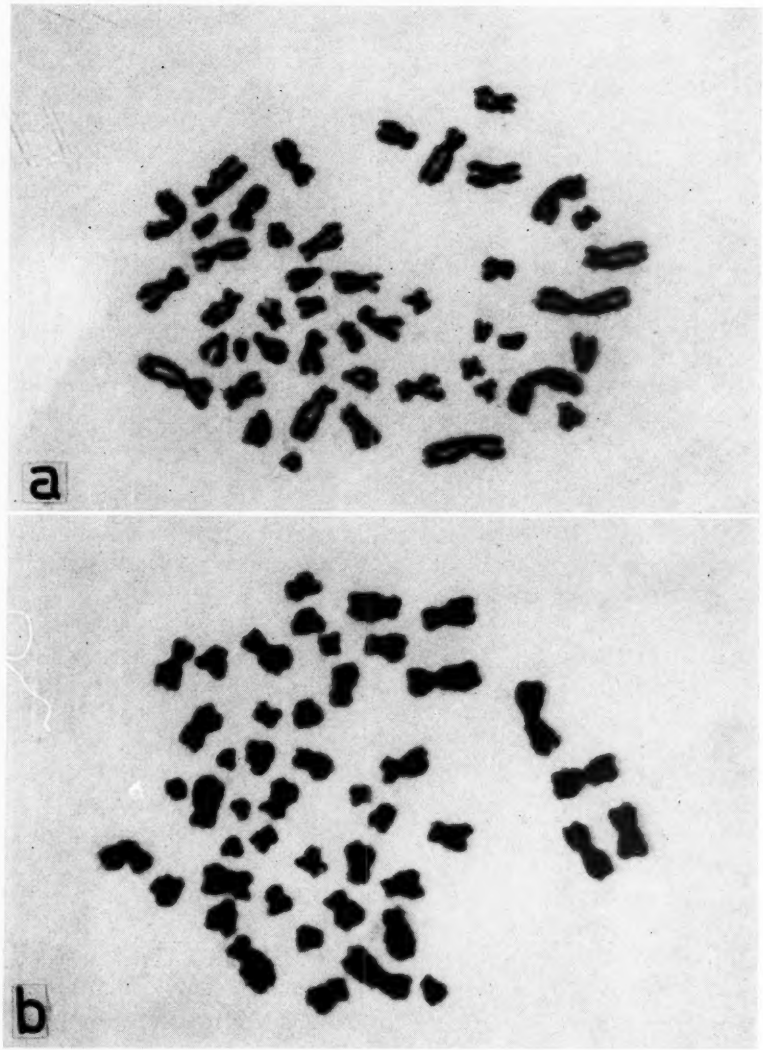


Fig. 1. Colchicine-metaphases of human embryonic lung fibroblasts grown *in vitro*. *a*: early metaphase, *b*: full metaphase. The two cells are from embryos 2 and 3 (Table 1), respectively. — ×2300.

CHROMOSOME MORPHOLOGY

Some data on the chromosome morphology of the 46 human chromosomes will be communicated here. The detailed idiogram analysis will be postponed, however, until we are able to study individuals of known sex, the sex of the present embryos being unknown. The comparative study of germline chromosomes in spermatogonial mitoses constitutes an urgent supplement to the present work.

In Fig. 2 four cells are analysed ranging from late prophase (*a*) to late c-metaphase (*d*). The chromosomes of metaphases with moderate colchicine contraction vary in length between 1 and 8 μ (Fig. 2 *b*), but the entire range of variation of Fig. 2 is from 1 to 11 μ. The chromosome morphology is roughly concordant with the observations of earlier workers, as, for instance, the idiogram of HSU (1952). The chromosomes may be divided into three groups: M chromosomes (median-submedian centromere; index long arm : short arm 1—1,9), S chromosomes (subterminal centromere; arm index 2—4,9), and T chromosomes (nearly terminal centromere; arm index 5 or more).

The M and S chromosomes are present in about equal numbers (twenty of each), while six T chromosomes are found. The classification of the three groups is arbitrary, of course, since gradual transitions of arm indices occur between the three groups. Certain submedian M chromosomes are hard to distinguish from some of the S chromosomes, and the most asymmetric S chromosomes approach the T group.

The chromosomes are easily arranged in pairs, but only certain of these pairs are individually distinguishable. Thus, the M chromosomes include the three longest pairs, which can always be identified. The two longest pairs are different: the second having a decidedly more asymmetric location of its centromere. The two or three smallest M pairs are also recognizable. Between the three longest and the three shortest pairs there are four intermediate pairs that cannot be individually recognized.

The S chromosomes are hardly identifiable, since they form a series of gradually decreasing length. The largest pair, however, is characteristic. Certain chromosomes were seen to have a small satellite on their short arms. Secondary constrictions, too, have been observed now and then, so that it may be hoped that the detailed morphologic study will lead to the identification of more chromosome pairs. The T chromosomes are recognizable; they constitute three pairs of middle-sized chromosomes. Unlike the mouse chromosomes, the human T chromosomes evidently have a small shorter arm.

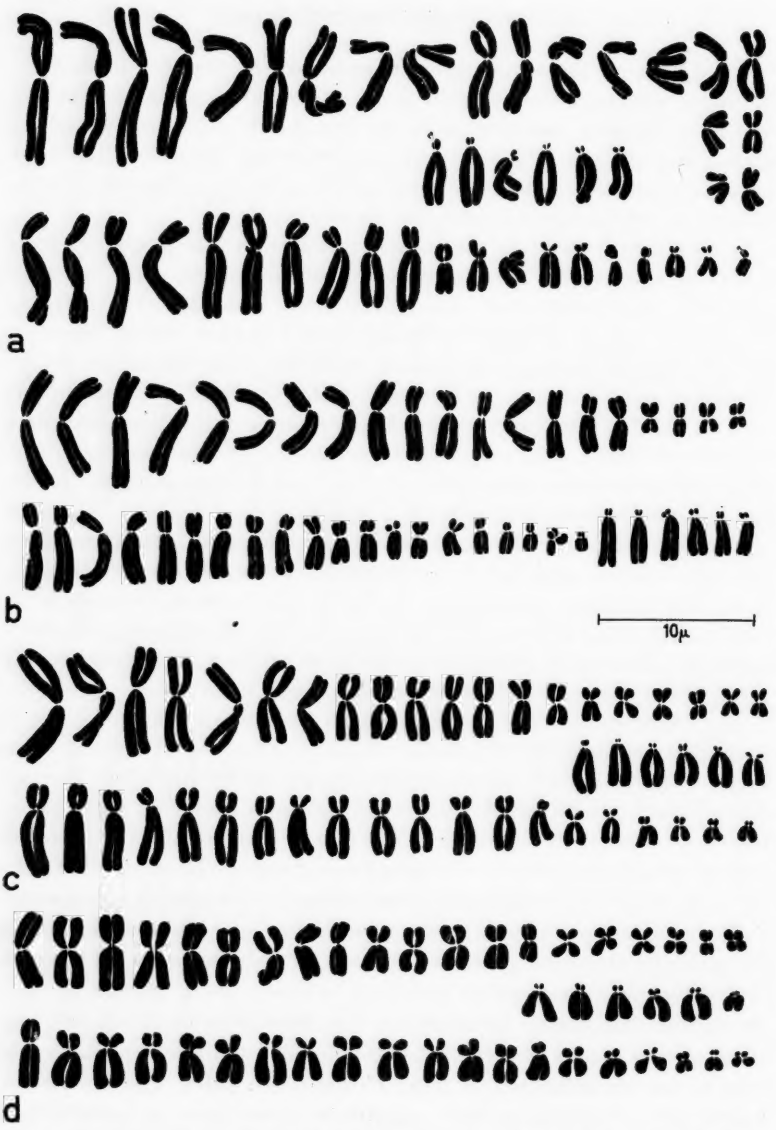


Fig. 2. Four idiogram analyses of human embryonic lung fibroblasts grown *in vitro*. The chromosomes have been grouped in three classes: M (top row), S (bottom row), and T (in between, except in *b*, where T is at the end of the S row). Within each class the chromosomes have been roughly arranged in diminishing order of size. — ×2400.

CONCLUSION

The almost exclusive occurrence of the chromosome number 46 in one somatic tissue derived from four individual human embryos is a very unexpected finding. To assume a regular mechanism for the exclusion of two chromosomes from the idiogram at the formation of a certain tissue is unlikely, even if this assumption cannot be entirely dismissed at this stage of inquiry. Our experience from one somatic tissue in mice and rats, *viz.*, regenerating liver, speaks against this assumption. The exact diploid chromosome set was always found in regenerating liver.

After the conclusion had been drawn that the tissue studied by us had 46 as chromosome number, Dr. EVA HANSEN-MELANDER kindly informed us that during last spring she had studied, in cooperation with Drs. YNGVE MELANDER and STIG KULLANDER, the chromosomes of liver mitoses in aborted human embryos. This study, however, was temporarily discontinued because the workers were unable to find all the 48 human chromosomes in their material; as a matter of fact, the number 46 was repeatedly counted in their slides. We have seen photomicrographs of liver prophases from this study, clearly showing 46 chromosomes. These findings suggest that 46 may be the correct chromosome number for human liver tissue, too.

With previously used technique it has been extremely difficult to make counts in human material. Even with the great progress involved in HSU's method exact counts seem difficult, judging from the photomicrographs published (HSU, 1952 and elsewhere). For instance, we think that the excellent photomicrograph of HSU published in DARLINGTON's book (1953, facing p. 288) is more in agreement with the chromosome number 46 than 48, and the same is true of many of the photomicrographs of human chromosomes previously published.

Before a renewed, careful control has been made of the chromosome number in spermatogonial mitoses of man we do not wish to generalize our present findings into a statement that the chromosome number of man is $2n=46$, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations.

Acknowledgements. — We wish to express our sincere thanks to the Swedish Cancer Society for financial support of this investigation, and to Dr. RUNE GRUBB for supplying us with tissue cultures.

SUMMARY

The chromosomes were studied in primary tissue cultures of human lung fibroblasts explanted from four individual embryos. In all of them the chromosome number 46 was encountered, instead of the expected number 48. Since among 265 mitoses counted all except 4 showed the number 46, this number is characteristic of the tissue studied. The possible bearing of this result on the chromosome number of man is discussed.

Institute of Genetics, Lund, January 26, 1956.

Literature cited

DARLINGTON, C. D. 1953. The facts of life. — London, 467 pp.

HSU, T. C. 1952. Mammalian chromosomes *in vitro*. — The karyotype of man. — J. Hered. 43: 167—172.

LEVAN, A. 1956. Chromosome studies on some human tumors and tissues of normal origin, grown *in vivo* and *in vitro* at the Sloan-Kettering Institute. — Cancer (in the press).

CYTO-TAXONOMICAL STUDIES IN THE GENUS LUZULA

II. HYBRIDIZATION EXPERIMENTS IN THE CAMPESTRIS-MULTIFLORA COMPLEX

By *HEDDA NORDENSKIÖLD*

INSTITUTE OF PLANT SYSTEMATICS AND GENETICS, ROYAL AGRICULTURAL COLLEGE OF SWEDEN, UPPSALA 7

INTRODUCTION

THE compatibility and hybrid fertility in crosses between the taxonomical units of the *campestris-multiflora* complex of the genus *Luzula* have been studied in material originating from different geographical regions of the northern hemisphere. The strains used for the investigation have chiefly been those previously described (NORDENSKIÖLD, 1949, 1951). Some collections, made at a later stage, have, however, been added to the ones already described. When a great many strains of one and the same species have been studied, as for instance with *L. campestris s. str.* or *L. multiflora* ($2n=36$), only a few representatives have been selected for hybridization experiments.

Of most interest for the investigation are two diploid species from Japan, *i. e.*, *L. capitata* (Mig.) NAKAI and *L. multiflora* v. *lutescens* SATAKE. A strain of *L. campestris* of Savoy, France, has been found with different stages of half-completed endonuclear polyploidy. Chromosome numbers from 12 to 18 have been counted in this collection (Fig. 1 *a*—*d*). Plants with such uneven chromosome number as 13 or 15 must be regarded as hybrids between those with an even chromosome number. I have not as yet had the opportunity of investigating meiosis in these plants, as they only produced a few flowers this summer (1955). Regarding the tetraploids I have received a strain of *L. multiflora* with $2n=28$ (Fig. 1 *e* and *f*) originating from the Gross Glockner Massive in the Alps, and, moreover, *L. congesta* (THUILL.) LEJ. ($2n=48$) is more abundantly represented. This last-mentioned species is the only true octoploid found in the complex. It is very often treated as a variety of *L. multiflora* (HYLANDER, 1953), although it seems to be rather distinctly characterized. It is not only distinguished by its congested head, but other

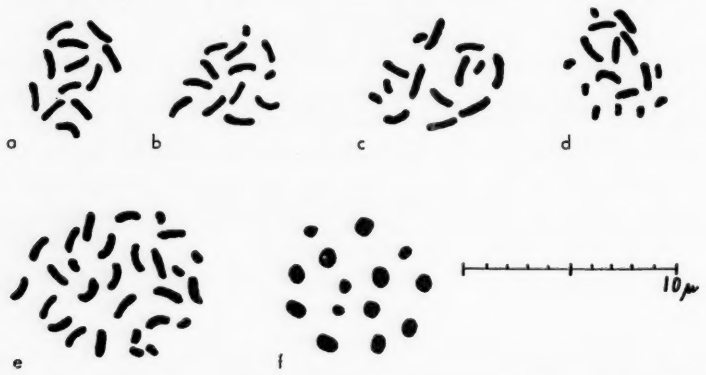


Fig. 1. (*a*—*d*). Somatic chromosomes of *L. campestris*, the strain originating from Savoy in France. (*a*) 12 chromosomes of AL-size, (*b*) 11 chromosomes of AL-size and 2 of BL-size, (*c*) 10 chromosomes of AL-size and 4 of BL-size, (*d*) 8 chromosomes of AL-size and 8 of BL-size; (*e*—*f*). Somatic and meiotic chromosomes of *L. multiflora*, the strain originating from Gross Glockner, Austria, (*e*) somatic metaphase plate with 20 chromosomes of AL-size and 8 of BL-size, (*f*) polar view of meiosis at first metaphase with 10 big AL-bivalents and 4 small BL-bivalents.

characteristics may be noted as, for instance, the occurrence of very few or often only a single stiff tiller that usually characterizes the octoploid *L. congesta*. Thus, I have found it most satisfactory to restrict the name *L. congesta* to the octoploid race only and to refer the tetra- and hexaploids to the collective species *L. multiflora*. My collections of the species *L. congesta* come mainly from Denmark, where it is common along the west coast of Jutland; on the dryer side of this peninsula, however, I did not find any localities for the species. A list of the strains of the *campestris-multiflora* complex, collected later than 1951, is shown in Table 1.

The localities, from which the material used in the present investigation originates, can be referred to certain geographical regions of the northern hemisphere. These regions could be assigned roughly as follows: Europe, Eastern North America, Arctic North America, Western North America, and Japan. In those geographical regions with the exception of Arctic North America, several species or taxonomical units of the *campestris-multiflora* complex have been found. The different species or varieties of a region are usually distinguished by different chromosome patterns, their chromosome numbers being built up by different types of true or endonuclear polyploidy (Table 2). To simplify

the tables the names of the taxonomical units examined are given by the species or variety names only. A complete nomenclature of these taxonomical units and references to their original descriptions have been previously given (NORDENSKIÖLD, 1951). In Table 2 the species or varieties originating from various geographical regions are referred to different levels of polyploidy. In addition, the chromosome numbers of each taxonomical unit are stated.

The species belonging to the diploid level of polyploidy usually possess 12 chromosomes. There are, however, some with deviating numbers, such as *sudetica* of Europe with 48 chromosomes, *orestra* of California with 20 or 22 chromosomes, and the collection of *campestris* of Savoy in France. These are the endonuclear polyploids, whose chromosome numbers seem to have been reached by some kind of fragmentation of the chromosomes and not by their multiplication (NORDENSKIÖLD, 1951). Thus, the raising of the chromosome numbers is not followed here by an increase of the chromatin mass or chromatid length of the nucleus, and consequently no change of level of polyploidy is attained. In the pictures of the somatic chromosomes of *orestra* it is easy to see the two large chromosomes of standard or AL size and the 20 »half-sized» chromosomes of BL size (Fig. 4 *a*), the same being true of the somatic chromosomes of the *campestris* strain originating from Savoy (Fig. 1 *a*—*d*). *Sudetica* has 48 chromosomes of a still smaller size, *i. e.*, the CL size (Fig. 3 *a*).

The majority of the species of the diploid level have, however, 12 chromosomes, and in one and the same geographical area there are two or three species or taxonomical units of this chromosome pattern. Such diploids of a certain geographical area always grow ecologically separated, even if they meet in some localities. In Scandinavia, for instance, we have *pallescens* with a northern and eastern distribution, requiring a rather moist habitat; and *campestris*, which has a more southern distribution, grows in sunny, dry habitats. In some localities they meet, however, and there natural hybrids occur. Similarly, in Eastern North America, we have two diploid species, one, *bulbosa*, with a more northern distribution, and another, *echinata*, with a southern distribution. Also in Japan we have two diploid species, *lutescens* and *capitata*, the latter demanding the warmer habitat. In Western North America we have *macrantha* and *laxa*, both of them described as subspecies of *L. comosa*. *Macrantha* grows in warmer and drier habitats than *laxa*. *Columbiana* is a 12-chromosomal species of Western North America growing in the subalpine regions, which species morphologically

TABLE 1. *Species, original localities and chromosome numbers*

Species	Localities of collection
L. campestris s. str.	Alingsås, Västergötland, Sweden
» »	Harstena, Östergötland, »
» »	Väddö, Upland, »
» »	Knutby, » »
» »	Kila, Södermanland, »
» »	» » »
» »	Markaryd, Småland, »
» »	Axelvold, Skåne, »
» »	Ribe, Jutland, Denmark
» »	Tisted, » »
» »	Lögstör, » »
» »	Gotland, Sweden
» »	Savoy, France
L. pallescens	Jönköping, Småland, Sweden
»	Bogesund, Upland, »
L. capitata	Itizyo-ij, Kyoto, Japan
»	Hyogo, »
»	Kyoto, »
L. multiflora v. *lutescens*	» »
» » »	Mt. Kongo, »
L. bulbosa	Windsor, Ont., Canada
L. echinata	Morris Co., N. J., U. S. A.
L. campestris v. *columbiana*	Chinquapin, Yosemite, Calif., U. S. A.
L. sudetica	Gross Glockner Massive, Austria
L. campestris v. *vallesiaca*	Vorarlberg, »
L. multiflora	Gross Glockner Massive, »
»	Koralpe, Steyria, »
»	Gross Glockner Massive, »
»	Massif Central, France
»	Windsor, Ont., Canada
»	Hangvar, Kullshage, Gotland, Sweden
»	Harstena, Östergötland, »
»	Axelvold, Skåne, »
»	Markaryd, Småland, »
»	Store mosse, » »
»	Near Fredensborg, Själland, Denmark
»	Middelfart, Fyn, »
»	Near Tisted, Jutland, »
»	» Lögstör, » »
»	Edge of Viborgs Hedeplantage, »
»	Near Silkeborg, Jutland, »
L. congesta	» Ribe, » »
»	South of Varde, » »
»	North » » » »
»	Cardiganshire, Wales
»	Coimbra, Portugal

of collections belonging to the campestris-multiflora complex.

Habitat	Collector	Material collected	No. of chromosome determined plants	$2n$
Meadow	Author	5 pl.	4	12
	Axel Olsson	3 »	2	12
Open pine wood	Author	16 »	4	12
Morain hillock	»	6 »	5	12
Opening in coniferous wood	»	9 »	4	12
Meadow	»	13 »	5	12
Open coniferous wood	»	12 »	7	12
Dry meadow	»	24 »	13	12
Heath	»	8 »	3	12
Peaty moor	»	12 »	1	12
Dry hillside	»	10 »	4	12
Sandy field	Bengt Pettersson	5 »	4	12
Dry meadow at 5000 ft., alt.	S. O. Björkman	seeds	8	12—18
Open pine wood	Author	2 »	1	12
Meadow	G. Turesson	4 »		
		seeds	5	12
	K. Matsumoto	»	4	12
	»	»	6	12
	»	»	3	12
	»	»	5	12
	A. Kalnins	»	6	12
	R. T. Clausen	»	2	12
At 7000 ft., alt.	G. B. van Schaack	»	5	12
Alpine meadow	Author	7 pl.	2	48
	Kiel, Bot. Garden	seeds	4	36
	M. Palm and S. Anderson	»	5	28
At 5200 ft., alt.	F. Widder 6	»	3	24
Alpine meadow	Author	23 pl.	6	24
	Besançon, Bot. Garden	seeds	3	24
			12	36
	A. Kalnins	»	6	24
Edge of pine wood	Bengt Pettersson	9 pl.	5	36
	Axel Olsson	4 »	4	36
Beech wood	Author	27 »	13	36
Edge of wood	»	18 »	7	36
Moor	»	17 »	9	36
Beech wood	»	13 »	4	36
» »	»	14 »	4	36
Swamp	»	17 »	4	36
Meadow at the beach	»	11 »	5	36
Open deciduous wood	»	11 »	5	36
Juncus swamp	»	10 »	4	36
Bank of a river	»	12 »	4	48
Salix swamp	»	4 »	4	48
Juncus swamp	»	8 »	4	48
At 2200 ft., alt.	S. O. Björkman	2 »	2	48
	Coimbra, Bot. Garden	seeds	4	48

TABLE 2. *The taxonomical units of the campestris-multiflora complex used in the investigation, their chromosome numbers, and their geographical origin.*

Region	Diploid level	Tetraploid level	Hexaploid level	Octoploid level
Eastern North America	*bulbosa* 2*n*=12 *echinata* 2*n*=12	*multiflora* 2*n*=24	*multiflora* 2*n*=36	
Europe	*pallescens* 2*n*=12 *campestris* 2*n*=12 *campestris* 2*n*=12—18 *sudetica* 2*n*=48	*multiflora* 2*n*=24 *multiflora* 2*n*=28 *vallesiaca* 2*n*=36	*multiflora* 2*n*=36	*congesta* 2*n*=48
Japan	*lutescens* 2*n*=12 *capitata* 2*n*=12	*multiflora* 2*n*=24		
Western North America	*laxa* 2*n*=12 *macrantha* 2*n*=12 *columbiana* 2*n*=12 *orestra* 2*n*=20, 22	*comosa* 2*n*=24		
Arctic North America		*groenlandica* 2*n*=24		

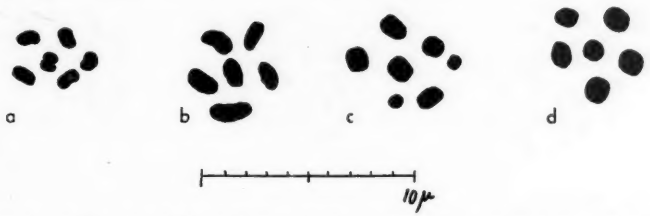


Fig. 2. Polar views of meiosis at first metaphase of (*a*) *campestris* showing 6 bivalents, (*b*—*c*) *campestris*×*macrantha*, (*b*) showing 6 bivalents and (*c*) showing 5 bivalents and 2 univalents, (*d*) *macrantha* showing 6 bivalents.

more nearly resembles *multiflora* or *sudetica* than *comosa* (St. JOHN, 1936).

The species or varieties of the tetraploid level of polyploidy have usually 24 chromosomes somatically. Only two of the systematical units studied, both originating from the Alps in Europe, have deviating chromosome numbers. The one, *vallesiaca* (*L. campestris* v. *vallesiaca* BEAUV. 1918), has 36 chromosomes (Fig. 5 *a* and *d*), 12 of standard or

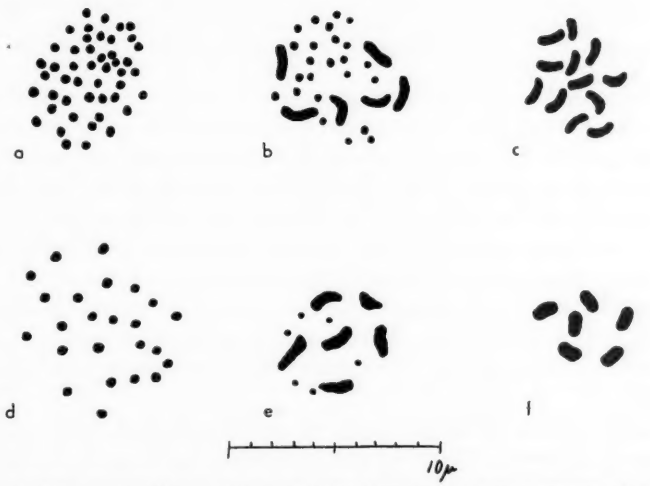


Fig. 3. Somatic chromosomes of (*a*) *sudetica* showing 48 chromosomes of CL-size, (*b*) *campestris*×*sudetica* showing 6 chromosomes of AL-size and 24 of CL-size, (*c*) *campestris* showing 12 chromosomes of AL-size; (*d*—*f*). Polar views of meiosis at first metaphase of (*d*) *sudetica* showing 24 CL-bivalents, (*e*) *campestris*×*sudetica* showing 6 big multi-associations, the size of an AL-bivalent, and 6 small CL-univalents, (*f*) *campestris* showing 6 AL-bivalents.

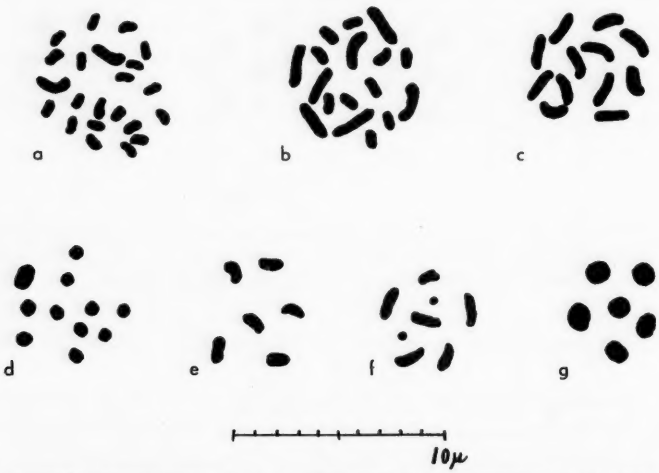


Fig. 4. Somatic chromosomes of (*a*) *orestra* with 2 chromosomes of AL-size and 20 of BL-size, (*b*) *orestra*×*macrantha* showing 7 chromosomes of AL-size and 10 of BL-size, (*c*) *macrantha* with 12 chromosomes of AL-size; (*d*—*g*). Polar views of meiosis at first metaphase of (*d*) *orestra* showing one big AL-bivalent and 10 small BL-bivalents; (*e*—*g*) *orestra*×*macrantha* showing (*e*) 6 big multi-associations, the size of an AL-bivalent, and (*f*) showing 6 multi-associations, the size of an AL-bivalent, and 2 small BL-univalents, (*g*) *macrantha* showing 6 AL-univalents.

AL size, and 24 of BL size. The other has 28 chromosomes (Fig. 1 *e* and *f*), two of the four chromosome sets having each 6 chromosomes of standard or AL size and the two remaining sets four chromosomes of the standard and four of the BL size. This latter taxonomical unit, like the majority of the species or varieties of the tetraploid level of polyploidy, has been referred to the collective species *L. multiflora*. Among the other tetraploids, *vallesiaca* of the Alps and *groenlandica* (*L. groenlandica* BÖCHER, 1950) of Arctic North America are both morphologically very similar to *multiflora*, whereas the West American species *comosa* is the only tetraploid which definitely does not belong to that collective species.

Species of the hexaploid level of polyploidy have been found in Europe and Eastern North America. Both these taxonomical units have been referred to *multiflora*; they have both the same chromosome numbers, *i. e.*, $2n=36$. Also found in Europe is the only true octoploid of the *campestris-multiflora* complex, *L. congesta*, with 48 chromosomes. It grows only in the Atlantic regions of Europe, from Norway in the north to Portugal in the south.

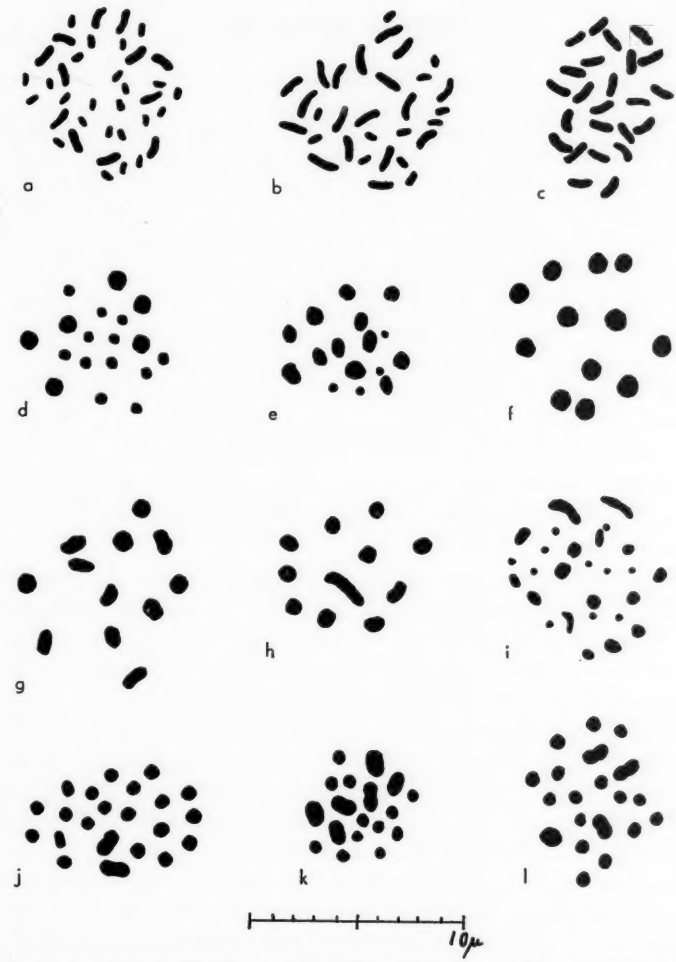


Fig. 5. (*a*—*c*). Somatic metaphases of (*a*) ***vallesiaca*** with 12 chromosomes of AL-size and 24 of BL-size, (*b*) ***vallesiaca*** × ***multiflora*** with 18 chromosomes of AL-size and 12 of BL-size, (*c*) ***multiflora*** with 24 chromosomes of AL-size; (*d*—*l*). Polar views of meiosis at first metaphase of (*d*) ***vallesiaca*** with 6 AL-bivalents and 12 BL-bivalents, (*e*) ***vallesiaca*** × ***multiflora*** with 12 multi-associations, the size of an AL-bivalent, and 4 BL-univalents, (*f*) ***multiflora*** of Europe with 24 AL-bivalents, (*g*) ***vallesiaca*** × ***multiflora*** with 12 multi-associations, the size of an AL-bivalent, (*h*) ***vallesiaca*** × ***multiflora*** with 10 multi-associations, the size of an AL-bivalent, and one big multi-association with the size of an AL-quadrivalent, (*i*) ***vallesiaca*** × ***groenlandica*** showing associations of different sizes from big ones of AL-quadrivalent-size to small univalents of BL-size, (*j*) ***multiflora*** of Eastern North America × ***multiflora*** of Europe showing 2 AL-bivalents and 20 AL-univalents, (*k*—*l*) ***multiflora*** of Japan × ***multiflora*** of Europe showing (*k*) 6 AL-bivalents and 12 AL-univalents and (*l*) showing 4 AL-bivalents and 16 AL-univalents.

TECHNIQUE

All strains of the *campestris-multiflora* complex, which have been cultivated and studied, have been found to be autogamous. In nature collected strains are, thus, very uniform, and such strains have been regarded as pure lines when they originate from single plants. In spite of the fact that all the strains studied are autogamous they are usually proterogynous, sometimes very strongly so. These facts facilitate the experiments. It is usually possible to fertilize the plants before the anthers have come out, especially when the plants are kept in a cool greenhouse during the flowering season. Consequently, no emasculation of the flowers has been necessary. Even if a late flower of the inflorescence were self-fertilized, it would usually be very easy to identify the non-hybrid plants as they are wholly similar to the mother strain. The hybrids, on the other hand, have always been intermediate between the parents. For this reason, therefore, it has been possible to carry out a great number of crosses.

The autogamous strains of the different species of the *campestris-multiflora* complex are usually fully fertile, *i. e.*, possessing a perfectly good pollen and complete fruit-setting ability. Thus, it has always been easy to obtain seeds of the strains used for the investigation. At first metaphase all the species of the complex form bivalents very regularly. This is not only the case with the diploids (Figs. 2 *a* and *d*, and 3 *f*), but also with the true or endonuclear polyploids collected in nature, where no regular occurrence of multivalents has ever been found at meiosis (Figs. 1 *f*, 3 *d*, 4 *d*, 5 *d*, and *f*). In the artificially produced tetraploids or hybrids multivalents may be found.

The study of the crosses has been principally made between species belonging to the same level of polyploidy. In addition, some crosses between species of different levels of polyploidy have been tried. The hybrids obtained have been investigated in the first as well as in the second generation. In the first hybrid generation the pairing of chromosomes during meiosis as well as the fruit-setting ability of the plants have been studied, in the second generation the viability and fertility of the F_2 plants have been investigated. The fruit-setting ability has been calculated as developed fruits in percentage of flowers of the inflorescence. Many fruits of plants with a reduced fertility have, however, only one or two seeds instead of the normally occurring three in the capsules. Thus, the fertility of the seeds in percentage of potential embryos of the flowers is usually lower than the fruit-setting ability.

When the fruit-setting ability is high, as holds good for the parental strains or for hybrids of certain combinations, the influence on fertility by environment is low and the fertility can then be measured fairly accurately. When, on the other hand, the fruit-setting ability is low, the environmental influence is much larger and the fertility can only then be given by an approximate estimation. This is the reason why the degree of fertility of the F_1 hybrids is given with 10 per cent accuracy for the more fertile plants but only with 25 per cent for the less fertile ones. The fertility of the plants of the second hybrid generation is, for the same reason, divided into only four classes. The viability of the F_2 generation has been calculated as adult plants in percentage of germinated seeds. When the figures in the tables are in brackets it indicates that the percentage is calculated on less than ten units.

INVESTIGATION OF HYBRIDS BETWEEN THE 12-CHROMOSOMAL SPECIES

Hybrids are easily formed between all the species of the diploid level of polyploidy, *i. e.*, all the species combinations tried have succeeded. The F_1 hybrids are generally vigorous, having an intermediate size between that of the parents. Only in a few strain combinations weak or abnormal F_1 hybrids are obtained. The number of F_1 hybrids found in a cross is to some extent a measure of the compatibility between the strains in question. On the other hand, some strains are more proterogynous than others and those species usually give a much lower number of non-hybrid plants than those which are less proterogynous when they are used as mother plants in a cross. When the amount of seed is obtained from the mother plant, 30 seeds from each cross have been planted. Each cross in the tables is usually made only once. In a few cases, however, the crosses have been repeated.

The results of the hybridization experiments of the 12-chromosomal species of the *campestris-multiflora* complex have been summarized in Tables 3—8. In each combination more than one strain or collection of a taxonomical unit have usually been employed. In some cases two strains of one and the same species have been crossed and investigated as a control. The manner in which the different properties given in the tables have been calculated is reported above (p. 16). In the column describing the pairing of chromosomes at first metaphase the percentage of cells with non-disturbed pairing is stated. When two figures are given for one strain combination in the column, two different F_1 plants

TABLE 3. *Results of the investigations of the hybrids in the first and lutescens (2n=12) and the other diploids*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
lutescens (Kyoto, Japan) × *capitata* (Kyoto, Japan)	18	99	90—100
lutescens (Mt. Kongo, Japan) × *campestris* (Södermanland 2, Sweden)	11	degenerated	70—80
lutescens (Kyoto, Japan) × *campestris* (Upland 21, Sweden)	30	partially degenerated	80—90
campestris (Upland 21, Sweden) × *lutescens* (Kyoto, Japan)	1	93	90—100
lutescens (Mt. Kongo, Japan) × *pallescens* (Södermanland 5, Sweden)	22	96	90—100
lutescens (Mt. Kongo, Japan) × *pallescens* (Tyrol, Austria)	13	90	70—80
pallescens (Tyrol, Austria) × *lutescens* (Kyoto, Japan)	7		90—100
» (Härjedalen, Sweden) × *lutescens* (Mt. Kongo, Japan)	1		0—25
lutescens (Mt. Kongo, Japan) × *bulbosa* (Quebec, Canada)	28	94	90—100
bulbosa (Quebec, Canada) × *lutescens* (Mt. Kongo, Japan)	20	93, 84	90—100
lutescens (Mt. Kongo, Japan) × *echinata* (Washington, D. C., U. S. A.)	15	85	25—50
echinata (Washington, D. C., U. S. A.) × *lutescens* (Mt. Kongo, Japan)	23	92	25—50
lutescens (Mt. Kongo, Japan) × *echinata* (Morris Co., N. J., U. S. A.)	22	degenerated	50—70
lutescens (Kyoto, Japan) × *echinata* (Morris Co., N. J., U. S. A.)	21		50—70
lutescens (Mt. Kongo, Japan) × *laxa* (Fraser Valley, B. C., Canada)	37	96	50—70
laxa (Fraser Valley, B. C., Canada) × *lutescens* (Mt. Kongo, Japan)	25	partially degenerated	50—70
lutescens (Kyoto, Japan) × *macrantha* (Siskiyou Co., Calif.)	13	97	50—70
» (Mt. Kongo, Japan) × *macrantha* (Stanford, Calif.)	2	77	50—70
macrantha (Marin Co., Calif.) × *lutescens* (Mt. Kongo, Japan)	2	degenerated	70—80
» (Vancouver Isl., Canada) × *lutescens* (Mt. Kongo, Japan)	hybrid seeds did not germinate		
macrantha (Monterey Co., Calif.) × *lutescens* (Kyoto, Japan)	0		
lutescens (Mt. Kongo, Japan) × *columbiana* (Tuolumne Co., Calif.)	38	96, 98	90—100
columbiana (Tuolumne Co., Calif.) × *lutescens* (Mt. Kongo, Japan)	0		
columbiana (Tuolumne Co., Calif.) × *lutescens* (Kyoto, Japan)	1		50—70
» (Tulare Co., Calif.) × *lutescens* (Mt. Kongo, Japan)	2	77, 86	50—70

[1] Seeds badly developed.

second generations of the cross combinations tried between the Japanese of the campestris-multiflora complex

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-fruiting F_2 plants	No. of white F_2 seedlings
		0—25	26—50	51—75	76—100			
98	91	—	3	53	44	36	4	—
18[1]	100	—	—	—	—	—	4	—
95	95	9	28	42	21	75	10	3
93	89	7	30	38	25	40	8	—
97	98	6	26	37	31	51	2	—
96	100	—	12	32	56	25	—	—
90	92	—	—	8	92	25	—	—
73	(13)	—	—	(100)	—	1	—	—
89	100	—	6	35	59	17	—	—
62	76	—	7	14	79	28	—	—
(100)	(100)	—	—	(50)	(50)	4	—	—
27[1]	(75)	—	—	(50)	(50)	2	1	—
85	78	2	27	44	27	63	8	—
93	89	5	11	54	30	57	18	—
87	85	12	28	49	11	67	6	2
no progeny studied								
100	100	2	17	51	30	47	9	—
100	94	4	19	40	37	27	1	—
87	—	92	8	—	—	13	—	—
92	73	4	26	45	25	97	14	13
95	53	(12)	(25)	(50)	(13)	8	2	1
91	90	16	22	38	24	60	6	2

TABLE 4. *Results of the investigations of the hybrids in the first and capitata (2n=12) and other diploids*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
capitata (Kyoto, Japan) × *campestris* (Upland 1, Sweden) ...	29	90	50—70
» (» ») × » (» 21, ») ...	26	degenerated	50—70
campestris (Upland 9, Sweden) × *capitata* (Kyoto, Japan)	4		50—70
capitata (Kyoto, Japan) × *pallescens* (Tyrol, Austria)	27	100	90—100
pallescens (Tyrol, Austria) × *capitata* (Kyoto, Japan)	5		70—80
» (Medelpad, Sweden) × *capitata* (Kyoto, Japan)	19	85, 96	80—90
capitata (Kyoto, Japan) × *bulbosa* (Quebec, Canada)	27	95, 93	80—90
bulbosa (Quebec, Canada) × *capitata* (Kyoto, Japan)	1		80—90
capitata (Kyoto, Japan) × *echinata* (Washington, D.C., U.S.A.)	5	95	25—50
echinata (Philadelphia, Penn., U.S.A.) × *capitata* (Kyoto, Japan)	2		
» (Morris Co., N. J., U. S. A.) × *capitata* (Kyoto, Japan)	15	88	50—70
capitata (Kyoto, Japan) × *laxa* (Fraser Valley, B. C., Canada)	22	92, 87	50—70
laxa (Fraser Valley, B. C., Canada) × *capitata* (Kyoto, Japan)	23	degenerated 98	70—80
capitata (Kyoto, Japan) × *macrantha* (Monterey Co., Calif.) ..	7	(F_1 abnormal)	25—50
» (» ») × » (Vancouver Isl., Canada)	16	degenerated 99	25—50
macrantha (Vancouver Isl., Canada) × *capitata* (Kyoto, Japan)	24	72	50—70
» (Stanford, Calif.) × *capitata* (Kyoto, Japan)	0		
columbiana (Tuolumne Co., Calif.) × *capitata* (Kyoto, Japan) ..	4	83	90—100
capitata (Kyoto, Japan) × *columbiana* (Tuolumne Co., Calif.) ..	15	89	90—100
columbiana (Tulare Co., Calif.) × *capitata* (Kyoto, Japan)	20		25—50
capitata (Kyoto, Japan × *columbiana* (Tulare Co., Calif.)	20	85	25—50

have been investigated. Usually 100 cells have been studied in each hybrid plant. At meiosis of the diploid hybrids the pairing of chromosomes is in general fairly regular. The cells with »disturbed pairing» usually contain only one or two unpaired »bivalents» (Fig. 2 *c*). On the other hand, the pollen mother cells are sometimes degenerated before first metaphase or the bivalents at first metaphase are clustered together; in such cases no investigation of meiosis can be performed. Such a degeneration of the pollen mother cells is very often connected with disturbances in the development of the embryo sac. Often the mem-

second generations of the cross combinations tried between the Japanese of the campestris-multiflora complex.

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-fruiting F_2 plants	No. of white F_2 seedlings
		0—25	26—50	51—75	76—100			
93	92	57	22	7	14	28	6	1
66	67	31	16	37	16	19	5	—
85	90	11	45	33	11	36	18	3
94	74	3	10	18	69	39	2	—
98	77	4	16	30	50	54	10	—
91	78	4	1	23	72	79	—	4
90	67	6	17	34	43	35	1	—
no progeny studied								
83	96	8	38	41	13	24	1	—
83	67	6	41	35	18	17	5	—
96	83	—	28	41	31	39	3	—
97	97	14	30	30	26	57	—	—
94	94	1	27	43	29	70	14	—
88	100	—	5	74	21	19	3	—
88	—	(12)	(38)	(13)	(37)	8	12	—
100	88	28	28	32	12	78	13	—
93	96	14	17	34	35	91	4	5
98	92	14	46	20	20	35	—	1
96	—	6	29	30	35	17	—	1
93	—	8	24	40	28	25	5	7

brane of the embryo sac seems to be weakly developed. In such F_1 plants the fruit-setting ability is more or less reduced.

In Table 3 the results of all the cross combinations with the Japanese species *L. multiflora* v. *lutescens* have been stated. In the combinations with *lutescens* hybrids are usually easily obtained. An exception must be made, however, regarding certain strain combinations of *lutescens*×*macrantha* and *lutescens*×*columbiana*, where the hybrids seem to be produced with more difficulty. Here, hybrid seeds of one strain combination, that between *macrantha* of Vancouver Island and *lutescens*

of Mt. Kongo, failed to germinate. In the combination *macrantha* of Monterey Co. and *lutescens* of Kyoto no hybrid seeds were obtained, a fact which might, however, depend on environmental circumstances. In the combination with *lutescens* and *columbiana* there is a tendency for a certain difference to appear in the reciprocal crosses — if significant, however, is not possible to say. The cross with *lutescens* as mother seems to be easier to perform than the reciprocal one. The fruit-setting ability of the F_1 hybrid is good in the combination with *capitata*, *i. e.*, the species from the same geographical region as *lutescens*; and the same is true of the combinations with *campestris* and *pallescens* of Europe as well as of *bulbosa* of Eastern North America. A reduced fertility of the F_1 hybrids is noticeable, however, in the combinations with the West American species *macrantha* and *laxa* and with the East American species *echinata*. In the combination with *columbiana* of Western North America one strain combination has given good fruit-setting ability but the other a low fertility. Bivalent formation at meiosis of the F_1 hybrid is usually good. On the other hand, the meiosis of the hybrids is in some cases disturbed by degeneration of the pollen mother cells. When degeneration is strong the chromosomes fail to stain and the spindle is disturbed; with a less strong degeneration the bivalents cluster together. In both cases it is impossible to study meiosis. Degeneration of meiosis is only found in certain strain combinations of the species combinations given in the table.

In the second hybrid generation the germination ability of the seeds is usually good. The same is also the case with the viability of the F_2 plants. Here a low viability of the second hybrid generation is usually connected with a high number of white seedlings in a certain cross combination, the white seedlings having been counted as non-viable plants. In the crosses with *lutescens* white seedlings are found to a high degree in one strain combination with *columbiana*. The F_2 progenies often hold plants with different fruit-setting ability, ranging from wholly fertile to completely sterile individuals. Different strain combinations of one and the same species combination give sometimes deviating results as to the fertility of their F_2s. The species combinations giving the best fertility in F_2 are those with *pallescens* and *bulbosa*.

The next Table (4) shows the results of the hybridization experiments with the other Japanese diploid species *L. capitata*. In the combinations with *capitata* all the crosses easily succeeded, not even excepting the West American species. The strain combination *capitata*

of Kyoto with *macrantha* of Monterey Co., Calif., gave, however, somewhat abnormal F_1 plants with crumbled leaves, a property which seems to originate from the *macrantha* strain (see below p. 32). Here, the fruit-setting ability of the F_1 hybrid is in many combinations lower than in the combinations with *lutescens* shown in Table 3. It is only the hybrids with the European *pallescens* and the East American *bulbosa* which have a relatively good seed-setting ability, *i. e.*, more than 75 per cent. One of the strain combinations with *columbiana* of Western North America has, however, a surprisingly high fertility. It may be pointed out that it is the same strain, that from Tuolumne Co., which gave the high fertility in the crosses with *lutescens*. Here the difference between two strain combinations of the same species combination is striking. The pairing of chromosomes at meiosis is usually good, so far as it has been possible to study this. In many combinations, however, the bivalents are clustered together at first metaphase, or the pollen mother cells are already degenerated before that stage. In the second hybrid generation the germination percentage as well as the viability are good in these hybrid combinations with *capitata*. The fruit-setting ability of the F_2 plants is the highest for the combinations with *pallescens* and *bulbosa*. In the other combinations it is rather low. White seedlings in the F_2 are found in the combinations with *columbiana*, and in some strain combinations with *pallescens* and *campestris*.

Table 5 shows the results of the crosses with the European *L. pallescens*. In the earlier Tables (3 and 4) it is shown that the hybrid combinations with this species have throughout given highly fertile plants in both first and second generations. In Table 5 we see that the formation of F_1 hybrids with *pallescens* is easily made in all combinations except the one with *echinata* of Eastern North America; and perhaps some reduction of compatibility is revealed in the combination with the West American *laxa*. In the last-mentioned combination, one of the crosses has given only one very weak F_1 hybrid. Pairing of chromosomes at first metaphase is good in the F_1 hybrids with the European *campestris* and the East American *bulbosa*, as well as in the ones with the two Japanese species *capitata* and *lutescens* (Tables 3—4). In the F_1 hybrids between *pallescens* and the West American species the pairing of chromosomes at first metaphase is significantly reduced. In the second hybrid generation the germination of the seeds is generally good and this is also true of the viability of the F_2 plants with the exception of those combinations giving a high percentage of white seedlings and one strain combination with *echinata*. The cross combination in this

TABLE 5. *Results of the investigations of the first and second hybrid (2n=12) and other diploids of*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
pallescens (Tyrol, Austria) × *pallescens* (Medelpad, Sweden) ..	6		90—100
» (Medelpad, Sweden) × *pallescens* (Tyrol, Austria) ..	7	100	90—100
pallescens (Medelpad, Sweden) × *campestris* (Upland 6, Sweden)	7		
» (Tyrol, Austria) × *campestris* (Upland 6, Sweden) ..	7	98	80—90
campestris (Upland 6, Sweden) × *pallescens* (Härjedalen, Sweden)	15	91, 98	70—80
campestris (Västergötland, Sweden) × *pallescens* (Tyrol, Austria)	5		80—90
bulbosa (Quebec, Canada) × *pallescens* (Härjedalen, Sweden) ..	20	97	90—100
pallescens (Medelpad, Sweden) × *bulbosa* (Quebec, Canada) ..	11		90—100
» (Tyrol, Austria) × *bulbosa* (Quebec, Canada)	3		90—100
bulbosa (Quebec, Canada) × *pallescens* (Tyrol, Austria)	15		90—100
pallescens (Medelpad, Sweden) × *echinata* (Washington, D. C., U. S. A.)	2		0—25
pallescens (Härjedalen, Sweden) × *echinata* (Washington, D. C., U. S. A.)	7	86	25—50
echinata (Washington, D. C., U. S. A.) × *pallescens* (Sweden) ..	1		
pallescens (Tyrol, Austria) × *echinata* (Washington, D. C., U. S. A.)	1	degenerated	
echinata (Washington, D. C., U. S. A.) × *pallescens* (Tyrol, Austria)	25		50—70
echinata (Morris Co., N. J., U. S. A.) × *pallescens* (Tyrol, Austria)	0		
pallescens (Härjedalen, Sweden) × *laxa* (Fraser Valley, B. C., Canada)	1	(F_1 very weak)	
laxa (Fraser Valley, B. C., Canada) × *pallescens* (Härjedalen, Sweden)	5		50—70
laxa (Fraser Valley, B. C., Canada) × *pallescens* (Medelpad, Sweden)	5	41, 20	
laxa (Fraser Valley, B. C., Canada) × *pallescens* (Tyrol, Austria)	2	degenerated	80—90
pallescens (Tyrol, Austria) × *laxa* (Fraser Valley, B. C., Canada)	2		80—90
pallescens (Härjedalen, Sweden) × *macrantha* (Marin Co., Calif.)	2	39	70—80
macrantha (Marin Co., Calif.) × *pallescens* (Härjedalen, Sweden)	1		90—100
» (» » ») × » (Tyrol, Austria)	13	41	50—70
pallescens (Tyrol, Austria) × *macrantha* (Marin Co., Calif.) ...	20	69, 55	50—70
macrantha (Siskiyou Co., Calif.) × *pallescens* (Härjedalen, Sweden)	4	67	0—25
pallescens (Härjedalen, Sweden) × *columbiana* (Tuolumne Co., Calif.)	20		50—70

generations in the cross combinations between the European pallescens the campestris-multiflora complex.

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants	No. of white F_2 seedlings
		0–25	26–50	51–75	76–100			
97	100	—	—	—	100	23	—	—
98	100	—	—	4	96	30	—	—
90	94	9	16	40	35	140	9	4
96	83	10	35	32	23	62	5	4
95	80	19	20	31	30	118	13	8
63	—	—	16	24	60	37	1	1
97	93	—	—	—	100	79	—	—
97	100	—	—	—	100	29	—	—
no progeny studied								
100	93	—	—	—	100	28	—	—
no progeny studied								
88	90	(100)	—	—	—	6	17	2
50	89	(89)	(11)	—	—	9	6	—
no progeny studied								
68	48	47	27	13	13	15	19	—
no progeny studied								
100	96	13	17	49	21	23	—	—
98	90	14	37	42	7	62	—	—
87	96	19	47	24	10	21	1	—
100	97	0	47	32	21	19	3	—
94	—	3	10	39	48	31	—	—
96	—	23	23	23	31	13	—	—
86	100	45	15	27	13	53	—	—
77	87	35	25	28	12	40	—	—
90	94	12	41	35	12	34	3	—
98	—	14	16	47	23	57	4	—

TABLE 6. *Results of the investigations of the first and second hybrid pestris s. str. (2n=12) and other diploids*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
campestris (Leeds, England) × *campestris* (Upland 21, Sweden)	10 ±		90—100
» (» ») × » (Småland, Sweden) ..	10 ±		90—100
bulbosa (Quebec, Canada) × *campestris* (Upland 6, Sweden) ..	7	92	70—80
campestris (Upsala, Sweden) × *echinata* (Washington, D. C., U. S. A.)	4		90—100
campestris (Upsala, Sweden) × *echinata* (Philadelphia, Penn., U. S. A.)	8		90—100
echinata (Washington, D. C., U. S. A.) × *campestris* (Upsala, Sweden)	11	F_1 plants weak	
campestris (Upland 6, Sweden) × *laxa* (Fraser Valley, B. C., Canada)	11	87	50—70
laxa (Fraser Valley, B. C., Canada) × *campestris* (Upsala, Sweden)	9	74	50—70
campestris (Upland 6, Sweden) × *macrantha* (Marin Co., Calif.)	9	85	50—70
» (» » ») × » (» » »)	3	F_1 very weak	
» (Västergötland, Sweden) × *macrantha* (Marin Co., Calif.)	22	88	50—70
campestris (Upland 21, Sweden) × *macrantha* (Monterey Co., Calif.)	1	96	50—70
macrantha (Marin Co., Calif.) × *campestris* (Upland 6, Sweden)	0		
» (Stanford, Calif.) × *campestris* (Upland 6, Sweden) ..	17	80	25—50
campestris (Leeds, England) × *columbiana* (Tulare Co., Calif.)	32	78	50—70
columbiana (Tulare Co., Calif.) × *campestris* (Upland 6, Sweden)	8	F_1 giving no flowers	

Table (5) giving the most remarkable result seems to be the one between *pallescens* and the East American species *bulbosa*. Between those two species no barrier of sterility seems to exist, neither in the first nor in the second generation.

Table 6 gives the crossing results of the combination with the European species *L. campestris s. str.* The F_1 hybrids in Table 6 are usually easily formed. On the other hand, strain combinations in the crosses with, for instance, *echinata*, *macrantha*, or *columbiana*, give hybrids which are abnormal or weak. The fertility of the F_1 plants is good in the combinations with *bulbosa* and *echinata* and this is also the case in

generations in the cross combinations between the European L. campestris-multiflora complex.

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants	No. of white F_2 seedlings
		0—25	26—50	51—75	76—100			
100	78	—	—	9	91	35	1	—
100	72	—	—	5	95	38	5	—
98	91	8	25	45	22	60	4	—
100	92	14	21	23	42	43	—	—
98	88	24	21	15	40	34	—	—
77	30	(17)	(66)	(17)	—	6	—	1
65	54	(29)	(57)	(14)	—	7	—	—
74	63	46	47	7	—	15	3	—
83	85	72	19	3	6	32	3	1
no progeny studied								
no progeny studied								
(71)	(20)	(100)	—	—	—	1	—	—
85	83	40	50	10	—	10	2	—
78	83	18	62	17	3	34	—	—

the previously described combinations with *lutescens* (Table 3), *capitata* (Table 4), or *pallescens* (Table 5). In the combinations with the West American species the fertility is, however, significantly reduced. In the second hybrid generation seed germination and viability of the F_2 plants are good for all combinations except for the ones with the West American species, and the same holds good for the fertility of the F_2 plants. In the crosses with *macrantha* the vitality of the F_2 plants in the combinations studied is, moreover, very weak.

Table 7 gives the hybridization results concerning the two East American diploid species, *L. bulbosa* and *L. echinata*, in combination

TABLE 7. *Results of the investigations of the first and second hybrid species bulbosa (2n=12) or echinata (2n=12) and*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
bulbosa (Quebec, Canada) × *echinata* (Philadelphia, Penn., U. S. A.)	8	94	70—80
bulbosa (Quebec, Canada) × *echinata* (Washington, D. C., U. S. A.)	4		
echinata (Washington, D. C., U. S. A.) × *bulbosa* (Quebec, Canada)	35	98, 99	90—100
bulbosa (Quebec, Canada) × *laxa* (Fraser Valley, B. C., Canada)	10	68 partially degenerated	50—70
» (» ») × *macrantha* (Monterey Co., Calif.) (F_1 abnormal)	5	57	50—70
macrantha (Monterey Co., Calif.) × *bulbosa* (Quebec, Canada)	14	F_1 giving no flowers	
bulbosa (Quebec, Canada) × *macrantha* (Vancouver Isl., Canada)	1	81, 84	70—80
columbiana (Tuolumne Co., Calif.) × *bulbosa* (Quebec, Canada)	9	71	50—70
echinata (Washington, D. C., U. S. A.) × *echinata* (Morris Co., N. J., U. S. A.)	2		90—100
echinata (Philadelphia, Penn., U. S. A.) × *laxa* (Fraser Valley, B. C., Canada)	hybrid seeds did not germinate		
echinata (Washington, D. C., U. S. A.) × *laxa* (Fraser Valley, B. C., Canada)	9	94, 85	25—50
laxa (Fraser Valley, B. C., Canada) × *echinata* (Morris Co., N. J., U. S. A.)	4	80	80—90
echinata (Philadelphia, Penn., U. S. A.) × *macrantha* (San Mateo Co., Calif.)	20	98 partially degenerated	
echinata (Washington, D. C., U. S. A.) × *macrantha* (Marin Co., Calif.)	2		0—25
echinata (Morris Co., N. J., U. S. A.) × *macrantha* (Berkeley, Calif.)	1	F_1 weak	50—70
echinata (Morris Co., N. J., U. S. A.) × *macrantha* (Monterey Co., Calif.)	4	F_1 abnormal	25—50
macrantha (Marin Co., Calif., U. S. A.) × *echinata* (Philadelphia, Penn., U. S. A.)	4		
macrantha (Vancouver Isl., Canada) × *echinata* (Washington, D. C., U. S. A.)	3	83 partially degenerated	50—70
columbiana (Tuolumne Co., Calif.) × *echinata* (Washington, D. C., U. S. A.)	3		50—70
columbiana (Tulare Co., Calif.) × *echinata* (Washington, D. C., U. S. A.)	6		

generations in the cross combinations between the two East American other diploids of the campestris-multiflora complex.

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants	No. of white F_2 seedlings
		0—25	26—50	51—75	76—100			
91	100	44	39	17	—	18	2	—
no progeny studied								
96	86	—	4	20	76	114	37	—
95	97	21	28	37	14	95	6	—
80	92	18	36	33	13	39	1	—
87	92	10	29	39	22	41	7	1
87	85	21	45	31	3	33	3	—
90	100	—	—	13	87	68	14	—
89	51	25	42	33	—	12	5	1
93	82	2	37	24	37	51	38	—
72	89	63	32	—	5	19	7	—
64	(66)	(33)	(34)	(33)	—	3	1	—
45	—	—	—	(33)	(67)	6	—	—
76	67	9	9	27	55	22	1	—
50	(63)	—	(25)	(50)	(25)	4	1	—
93	76	15	17	37	31	41	7	—
94	73	destroyed during winter						

TABLE 8. *Results of the investigations of the first and second hybrid American species macrantha, laxa, and colum-*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Per cent M_I cells with full pairing	Fruit-setting ability. Per cent
macrantha (Vancouver Isl., Canada) × *macrantha* (Monterey Co., Calif.)	9	badly developed flowers	
macrantha (Vancouver Isl., Canada) × *macrantha* (Stanford, Calif.)	10 ±		90—100
macrantha (San Mateo Co., Calif.) × *macrantha* (Monterey Co., Calif.)	10 ±		90—100
macrantha (Stanford, Calif.) × *macrantha* (Monterey Co., Calif.)	10 ±		90—100
macrantha (Marin Co., Calif.) × *laxa* (Fraser Valley, B. C., Canada)	10 ±	10	
macrantha (Vancouver Isl., Canada) × *laxa* (Fraser Valley, B. C., Canada)	12	90	90—100
macrantha (Stanford, Calif.) × *laxa* (Fraser Valley, B. C., Canada)	3		90—100
» (Siskiyou Co., Calif.) × *laxa* (Fraser Valley, B. C., Canada)	4		90—100
laxa (Fraser Valley, B. C., Canada) × *macrantha* (Marin Co., Calif.)	7	21	90—100
laxa (Fraser Valley, B. C., Canada) × *macrantha* (Vancouver Isl., Canada)	10 ±	100	90—100
macrantha (Stanford, Calif.) × *columbiana* (Tuolumne Co., Calif.)	0		
columbiana (Tulare Co., Calif.) × *macrantha* (Vancouver Isl., Canada)	14	97, 98	90—100
laxa (Fraser Valley, B. C., Canada) × *columbiana* (Tuolumne Co., Calif.)	25	97, 98	90—100
columbiana (Tuolumne Co., Calif.) × *laxa* (Fraser Valley, B. C., Canada)	10		90—100

with the other 12-chromosomal species. In the crosses with *bulbosa* and the other diploids the F_1 hybrids are easily obtained. The fertility of the F_1 plants as well as the pairing of chromosomes at first metaphase have been good for all combinations except for the ones with the West American species (Tables 3—7). In the corresponding crosses with the East American species *echinata* the compatibility is good except in one species combination, that with *pallescens* of Europe, where it is somewhat reduced (Table 5). The fruit-setting ability of the F_1 plants, on the

generations in the cross combinations between the three diploid West biana of the campestris-multiflora complex.

Second hybrid generation, F_2								
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants	No. of white F_2 seedlings
		0—25	26—50	51—75	76—100			
no progeny studied								
97	97	—	3	14	83	69	5	—
100	80	—	—	—	100	22	—	—
84	62	—	—	7	93	46	1	—
no progeny studied								
98	90	—	3	6	91	71	16	—
no progeny studied								
94	100	—	2	32	66	44	6	—
93	92	—	5	17	78	54	2	—
97	98	3	3	20	74	90	19	—
100	83	12	22	40	26	57	—	—
97	93	—	—	12	88	59	10	—
97	97	—	2	15	83	65	1	—

other hand, seems to be normal only in the combinations with *campestris* of Europe (Table 6) and *bulbosa* of Eastern North America (Table 7). It varies, however, very distinctly according to the strains used. If the *echinata* strain of Morris Co., N. J., is used in the combination, the F_1 hybrids are always more fertile than when one of the other two strains of Philadelphia or of Washington, D. C., are employed. The pairing of chromosomes at first metaphase in the combinations with *echinata* is usually fairly good, when meiosis does not show signs

of degeneration. In the combinations between the East and the West American species the fertility of the F_1 hybrids is throughout very low, and first metaphase is very often disturbed. In combinations with the *macrantha* strain of Monterey Co., California, the F_1 was abnormal in the crosses with both *bulbosa* and *echinata*; and in one strain combination between *echinata* of Philadelphia and *laxa* of Fraser Valley the hybrid seeds did not germinate.

Table 8 shows the relationship between the three diploid taxonomical units of Western North America. Here the seed-setting ability of the F_1 hybrids is always above 90 per cent. The pairing ability of chromosomes at first metaphase is good in the combinations between *L. campestris* v. *columbiana* and the two diploid varieties of *L. comosa*, *macrantha* and *laxa*. The hybrids between *laxa* and *macrantha* have, however, a very low pairing ability of the chromosomes during first metaphase in two of the strain combinations. The fixations are, however, very good, and no signs of degeneration can be seen. Moreover, the fertility of the F_1 plants is not affected at all. This low pairing ability of the chromosomes appears when two strains originating from distant localities are crossed, *i. e.*, a strain from California with one of British Columbia. In the cross combination where both strains originate from localities in the vicinity of each other, the pairing ability is normal. The fertility of the F_2 generation is fairly high in the combinations *laxa* and *macrantha* or *laxa* and *columbiana*; in the combination between *macrantha* and *columbiana* it is, however, distinctly reduced.

The F_1 hybrids described above have usually been of a normal size and intermediate between their parents. In some combinations, however, they are more or less abnormal. These abnormalities of the F_1 hybrid turn up in different ways, sometimes as very weak plants, sometimes as plants with crumbled leaves or abnormal inflorescences. In a few cases the combination seems to be lethal, giving non-germinating hybrid seeds. Such abnormal F_1 hybrids have only been demonstrated among the crosses of the species of the diploid level of polyploidy. Table 18 (p. 58) shows the occurrence of abnormalities in the different species combinations. Most frequently such abnormalities occur when the species *macrantha* is involved in a cross. Here, it is especially one strain, originating from Monterey Co. in California, which in many combinations with, for instance, *capitata*, *echinata* or *bulbosa* gives abnormal hybrids. Also in a combination with another strain of the same species, *macrantha* of Vancouver Island, the F_1 hybrid produced had badly developed inflorescences. When the Monterey strain is crossed

with other *macrantha* strains that originate from localities in the vicinity of Monterey — as the ones named »Stanford» or »San Mateo» —, the F_1 hybrids are, however, normal. There are also two other species which give abnormalities in more than one species combination. Those two species are *campestris* and *echinata* (Table 18).

HYBRIDS BETWEEN 12-CHROMOSOMAL DIPLOIDS AND ENDONUCLEAR POLYPLOIDS OF THE DIPLOID LEVEL

Among the species of the diploid level of polyploidy of the *campestris-multiflora* complex there are some endonuclear polyploids. Such species are *L. sudetica* of Europe with $2n=48$ (Fig. 3 *a* and *d*) and *L. orestra*, originating from the mountains of Western North America, with $2n=20$ or 22 (Fig. 4 *a* and *d*). Those endonuclear polyploids are easily crossed with the 12-chromosomal species of the diploid level.

Among the last collections of *L. campestris* studied a strain showing endonuclear polyploidy has also been found originating from Savoy in France. It shows varying chromosome numbers in the same collection, such as 12, 13, 14, 15, 16, 17, and 18 (Fig. 1 *a—d*). The chromosome numbers of the strain have been studied this year (1955) and the plants have, consequently, not been used in the crosses.

Crosses between *sudetica* and *campestris*, *pallescens*, *echinata*, *macrantha*, or *laxa* have been made, and in each species combination hybrids have been obtained (Table 9). The somatic chromosome pattern of the parents and the F_1 hybrid of the cross between *sudetica* and *campestris* is given in Fig. 3 *a—c*. In the F_1 hybrid it is easy to recognize the six large chromosomes of standard or AL size and the 24 small ones of CL size. All these hybrids between *sudetica* and the 12-chromosomal diploids were sterile and did not give any seeds. Meiosis of the F_1s has been studied at first metaphase, but only in the hybrids with *campestris* and *pallescens* is it possible to investigate the chromosome associations at first metaphase. The other three types of F_1 hybrids have their pollen mother cells degenerated already at an early stage.

In the hybrids between *sudetica* and *pallescens* or *campestris* — those in which meiosis could be investigated — it was possible to observe six large multi-associations built up on the six large AL chromosomes and a number of equal-sized very small chromosome bodies, which have been interpreted as univalents of CL size (Fig. 3 *e*). These facts indicate that the small chromosomes usually pair with the big ones but not with each other.

TABLE 9. *Results of the crosses between the endonuclear polyploid of the diploid level L. sudetica (2n=48) and the 12-chromosomal diploids of the campestris-multiflora complex.*

Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent
sudetica (Härjedalen, Sweden) × *campestris* (Södermanland 3, Sweden)	1	6 multi-associations + single univalents	sterile
» (» , ») × » (» 2, »)	23	6 multi-associations + single univalents	»
pallescens (Härjedalen, Sweden) × *sudetica* (Härjedalen, Sweden)	6	6 multi-associations + single univalents	»
sudetica (Härjedalen, Sweden) × *pallescens* (» , »)	7	6 multi-associations + single univalents	»
sudetica (Härjedalen, Sweden) × *macrantha* (Monterey Co., Calif.)	5	—	»
macrantha (Marin Co., Calif.) × *sudetica* (Härjedalen, Sweden)	0		
sudetica (Härjedalen, Sweden) × *laxa* (Fraser Valley, B. C., Canada)	20 ±	degenerated	»
laxa (Fraser Valley, B. C., Canada) × *sudetica* (Härjedalen, Sweden)	15 ±	»	»
sudetica (Härjedalen, Sweden) × *echinata* (Washington, D. C., U. S. A.)	15 ±	»	»

In a manner similar to that described for *sudetica* the hybrids between *orestra* (2*n*=22) and the 12-chromosomal diploids have been made. Hybrids have been produced with all the nine diploids used in the investigation (Table 10). The F_1 hybrids are partially fertile with a reduced fruit-setting ability, but seeds have been obtained from all the different hybrids. The F_1 plant has 17 chromosomes, 7 large ones of standard or AL size and 10 small ones of BL size (Fig. 4 *b*). Meiosis has been studied in the hybrids between *orestra* and the two West American species *macrantha* and *laxa*. During first metaphase six multi-associations were found and, in addition, sometimes none, sometimes one to several small univalents (Fig. 4 *e* and *f*). The six large associations must be built up on the six AL chromosomes from the 12-chromosomal diploid. The 11 chromosomes of *orestra* (one large and 10 small ones) seemed to pair with the six large chromosomes of the true diploid with only a few exceptions. The chromosomes were small and the fixation did not permit a more thorough investigation of the pairing.

The hybrid between *orestra* (2*n*=22) and *sudetica* (2*n*=48) has been obtained (Table 10), but it was completely sterile and in the buds studied the pollen mother cells were already degenerated before first metaphase.

The hybrids between *orestra* and *capitata* of Japan, *bulbosa* of Eastern North America as well as *campestris* of Europe, have also been studied in regard to their pollen mother cells, but the chromosomes were clustered together or the cells degenerated at first metaphase. Thus, the same phenomenon is demonstrated in these crosses as in the combinations between *sudetica* and the other diploids of the complex. When hybrids are made between species of the same geographical area meiosis of the hybrids may be studied; when, however, they are made between species originating from different geographical regions meiosis has been found to be degenerated at an early stage.

A second hybrid generation has been obtained from several of the hybrids between *orestra* and the other 12-chromosomal diploids (Table 10). Especially the hybrids with *pallescens* and *bulbosa* gave a surprising abundance of seeds, but germination and viability of the F_2 were low. The F_1 hybrids with *macrantha* unfortunately dropped many of their seeds before harvest, on account of which only a few seeds could be planted. The F_2 plants originating from the crosses with *laxa* were more fertile than the F_2 plants originating from the other combinations. A third generation was planted this year (1955) with seeds from the most fertile F_2 plants. It has already been easy to see in the young plants that the most vital combination is the one with *laxa*. Here,

TABLE 10. *Results of the crosses between the endonuclear polyploid of of the campestris-*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent
orestra (Mono Co., Calif.) × *sudetica* (Styria, Austria)	0		
orestra (Mono Co., Calif.) × *sudetica* (Härjedalen, Sweden)	10	degenerated	sterile
orestra (Mono Co., Calif.) × *lutescens* (Mt. Kongo, Japan)	6		0—25
lutescens (Kyoto, Japan) × *orestra* (Mono Co., Calif.)	8		0—25
orestra (Mono Co., Calif.) × *capitata* (Kyoto, Japan)	6	degenerated	25—50
orestra (Mono Co., Calif.) × *pallescens* (Medelpad, Sweden)	6	»	25—50
orestra (Mono Co., Calif.) × *campestris* (Västergötland, Sweden)	9	»	0—25
orestra (Mono Co., Calif.) × *bulbosa* (Quebec, Canada)	6		25—50
orestra (Mono Co., Calif.) × *echinata* (Philadelphia, Penn., U. S. A.)	3		0—25
orestra (Mono Co., Calif.) × *echinata* (Washington, D. C., U. S. A.)	3		0—25
orestra (Mono Co., Calif.) × *macrantha* (Marin Co., Calif.)	3	6 multi-associations + single univalents	25—50
orestra (Mono Co., Calif.) × *laxa* (Fraser Valley, B. C., Canada)	2	6 multi-associations + single univalents	25—50
columbiana (Tulare Co., Calif.) × *orestra* (Mono Co., Calif.)	2		25—50

the seeds germinated well and had a good viability but in the combinations with, for instance, *bulbosa* and *pallescens*, the seeds germinated very badly (about 50 per cent), and the viability of the seedlings was low.

the diploid level L. orestra (2n=22) and the 12-chromosomal diploids multiflora complex.

Second hybrid generation, F_2							
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants
		0—25	26—50	51—75	76—100		
67	80	(50)	—	(11)	(39)	8	—
(50)	(100)	(50)	(50)	—	—	2	—
86	(75)	(25)	(25)	—	(50)	4	1
70	60	71	15	14	—	14	1
no progeny studied							
76	79	44	30	15	11	27	—
no progeny studied							
(0)	—						
74	50	(25)	—	(25)	(50)	8	3
87	73	17	11	39	33	18	1
no progeny studied							

HYBRIDS BETWEEN SPECIES BELONGING TO THE TETRAPLOID LEVEL OF POLYPLOIDY

The majority of the taxonomical units belonging to the tetraploid level of polyploidy are referred to the collective species *L. multiflora.*

TABLE 11. *Results of the investigations of the first and second hybrid (2n=24) and the other tetraploids*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent
multiflora (Oze, Japan) × *multiflora* (Styria 11, Austria) (M_I: Fig. 5 *k*—*l*)	2	usually $4_{II}+16_I$, max. 7 biv.	0—25
multiflora (Oze, Japan) × *multiflora* (Styria 5, Austria)	11	» $3_{II}+18_I$, » 8 »	25—50
multiflora (Styria 5, Austria) × *multiflora* (Oze, Japan)	30	» $1_{II}+22_I$, » 2 »	0—25
multiflora (Oze, Japan) × *multiflora* (Mt. Washington, N. H., U. S. A.)	7	degenerated	0—25
multiflora (Oze, Japan) × *multiflora* (Franklin Co., Vt., U. S. A.)	19	usually $2_{II}+20_I$, max. 8 biv.	0—25
multiflora (Oze, Japan) × *comosa* (Tuolumne Co. 5, Calif.)	0		
multiflora (Oze, Japan) × *comosa* (Tuolumne Co. 7, Calif.)	0		
comosa (Tuolumne Co. 5, Calif.) × *multiflora* (Oze, Japan)	0		
multiflora (Oze, Japan) × *groenlandica* (James Bay, Canada)	36	usually $3_{II}+18_I$, max. 7 biv.	0—25
groenlandica (James Bay, Canada) × *multiflora* (Oze, Japan)	33	» $4_{II}+16_I$, » 8 »	0—10
multiflora (Oze, Japan) × *vallesiaca* (Tyrol 5, Austria)	20	high degree of pairing, partially degenerated	0—10
multiflora (Oze, Japan) × *vallesiaca* (Tyrol 13, Austria)	29		0—10
vallesiaca (Tyrol 13, Austria) × *multiflora* (Oze, Japan)	27	weak pairing, no degeneration	0—10
multiflora (Oze, Japan) × *t. pallescens* (Härjedalen, Sweden)	15	often 12_{II}, usually $11_{II}+2_I$, max. 3_{IV}	70—80

This species is represented in Europe, Eastern North America, and Japan. In Arctic North America *L. groenlandica* BÖCHER is a species which, morphologically, is very closely related to *L. multiflora* (BÖCHER, 1950). The same is the case with *L. campestris* v. *vallesiaca* BEAUV. of the Alps (BEAUVERD, 1918). *L. comosa* E. MEY. is the only tetraploid species which, morphologically, is distinctly dissimilar to *L. multiflora*.

generations in the cross combinations between the Japanese multiflora of the campestris-multiflora complex.

Second hybrid generation, F_2							
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants
		0—25	26—50	51—75	76—100		
(91)	(70)	(100)	—	—	—	5	2
60	53	20	47	20	13	15	2
95	53	30	24	29	17	29	2
(100)	(100)	(100)	—	—	—	1	2
(91)	(40)	(100)	—	—	—	3	3
90	52	14	39	22	25	28	3
(70)	(40)	(100)	—	—	—	1	—
(76)	(21)	(100)	—	—	—	5	—
(56)	(80)	(100)	—	—	—	1	3
91	95	(40)	(60)	—	—	5	14
92	83	5	22	22	51	45	4

The species belonging to the tetraploid level of polyploidy have all $2n=24$, except two taxonomical units, *i. e.*, *vallesiaca* with $2n=36$ and a strain of *multiflora* with $2n=28$. Both these endonuclear polyploids originate from the Alps in Europe. Apart from the natural tetraploids, an artificially made tetraploid strain of *L. pallescens* has been used in the experiments. Morphologically, this strain of tetraploid *pallescens* is

very similar to *multiflora*, especially to certain strains of North American origin. Species or varieties belonging to the tetraploid level of polyploidy are, with one exception, easy to hybridize with each other. The exception is the West American species *comosa*, which species in some combinations did not give any hybrids and in the other combinations gave hybrids only with difficulty.

Table 11 presents the results of the crosses between the Japanese *multiflora* and the other taxonomical units of the tetraploid level. All the crosses have easily succeeded except the one with *comosa*, which combinations have not given any hybrids at all. Meiosis of the F_1 hybrids is generally possible to study, complete degeneration occurring only in one strain combination with the East American *multiflora*. Meiosis of the tetraploids is much more difficult to study than that of the diploids. It has not been considered necessary to investigate more than 10 to 20 cells of each F_1 hybrid, as an adequate opinion of the process can be obtained by the study of a rather low number of cells. The highest degree of pairing of chromosomes is found in the hybrid with the artificially-made tetraploid *pallescens*. In this hybrid the two chromosome sets of *pallescens* must be homologous and able to pair. The other two sets originating from the Japanese *multiflora* seem here to be able to pair fairly regularly, as 12 bivalents are very common in the hybrid and up to three quadrivalents have been observed. In the F_1 hybrids between the natural tetraploids, bivalents are much more rare and quadrivalents never occur with regularity. In the hybrids with European *multiflora* or *groenlandica* three to four and with a maximum of 8 bivalents are usually found at first metaphase (Fig. 5 *k* and *l*). One of the strain combinations in the cross between the Japanese and European *multiflora* has, however, a very low degree of pairing, usually only $1_{II}+22_{I}$. In the hybrids between the Japanese and the East American *multiflora*, the pairing at first metaphase is also rather low in the strain combinations, which could be studied. Thus, *multiflora* of Japan acts as an autotetraploid in the crosses with the artificially-made tetraploid *pallescens*, but in hybrids with the natural tetraploids the chromosomes of the Japanese *multiflora* do not seem to be able to pair regularly. In the hybrid between the Japanese *multiflora* and the endonuclear polyploid *vallesiaca* the pairing at first metaphase is difficult to study. One strain combination had a partially degenerated meiosis, the other strain combination did not show any signs of degeneration but, on the other hand, a very low degree of pairing. Twenty-four to thirty chromosome bodies are found in the cells during first metaphase. These chromosome bodies

are of very varying size, the big ones being most likely built up by two to several chromosomes, and the small ones of varying sizes being probably univalents of AL and BL size.

The seed-setting ability of the F_1 hybrids, as set out in the table, is usually low. The only combination with a more fertile hybrid is the one with the artificially-made tetraploid *pallescens*. In many combinations a sufficient amount of seeds for a normal F_2 progeny is not obtained from one plant. The germination ability for the seeds harvested on the F_1 plants is fairly good but the viability of the young F_2 plants is usually low, *i. e.*, a high percentage of the plants died at an early stage. In the second generation the seed-setting ability is rather low, and when only a few seeds have been harvested on the F_1, the F_2 plants originating from these seeds are usually completely sterile. The cross combination with the most fertile F_2 is the one with the tetraploid *pallescens*. Among the cross combinations between natural tetraploids involving the Japanese *multiflora* there are only two combinations in the F_2 samples from which any fertile individuals occur. These two combinations are those with the European *multiflora* and *groenlandica* of Arctic North America.

In Table 12 are seen the hybridization results concerning the two geographical races of tetraploid *multiflora* originating from Europe and Eastern North America. These two taxonomical units, referred to the same collective species, show, however, a distinct barrier of sterility between them. At first metaphase of the F_1 hybrid the strain combination that is possible to study shows a low formation of bivalents (Fig. 5 *j*). The F_1 plants are almost sterile and the viability of the young F_2 plants is rather low. The F_2 progeny is generally sterile and only a few fertile plants are obtained. The East American race of the tetraploid *multiflora* is possible to hybridize with all the other tetraploids including the West American species *comosa*. This last-mentioned combination, however, gave hybrids only with difficulty, and the F_1 plants produced only a few seeds, from which no adult plants were obtained. Surprisingly enough the hybrids between the European *multiflora* and *comosa* seem to be easier to produce than the ones between the East American *multiflora* and *comosa*. The F_1 plants obtained between the European *multiflora* and *comosa* have a weak pairing of chromosomes during first metaphase and the hybrids are almost sterile. Some surviving F_2 plants are, however, obtained, and these show a low fertility. Another striking fact that emerges from the Table (12) is the different behaviour of the two *multiflora* races in their crosses with the species *groenlandica* of Arctic North America. Here, the hybrids between the East American

TABLE 12. *Results of the investigations of the first and second hybrid multiflora (2n=24) originating from Europe and Eastern North*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent
multiflora (Franklin Co., Vt., U. S. A.) × *multiflora* (Styria 5, Austria)	9		0—25
» (Caledonia Co., Vt., U. S. A.) × *multiflora* (» 9, »)	23	usually $1_{II}+22_I$, max. 5 biv. (Fig. 5 *j*)	0—25
» (Styria 5, Austria) × *multiflora* (Franklin Co, Vt., U. S. A.)	17	degenerated	0—25
multiflora (Mt. Washington, N. H., U. S. A.) × *comosa* (Vancouver Isl., Canada)	1	»	0—10
multiflora (Franklin Co., Vt., U. S. A.) × *comosa* (Tuolumne Co. 5, Calif.)	1		0—10
multiflora (Franklin Co., Vt., U. S. A.) × *comosa* (Vancouver Isl., Canada)	0		
multiflora (Styria 7, Austria) × *comosa* (Vancouver Isl., Canada)	5	usually $1_{II}+22_I$, max. 4 biv.	0—10
» (Caledonia Co., Vt., U. S. A.) × *groenlandica* (Hudson Bay, Canada)	15		sterile
groenlandica (Hudson Bay, Canada) × *multiflora* (Compton Co., Que., Canada)	25		»
groenlandica (Hudson Bay, Canada) × *multiflora* (Caledonia Co., Vt., U. S. A.)	5	degenerated	»
multiflora (Styria 5, Austria) × *groenlandica* (Hudson Bay, Canada)	16	usually $6_{II}+12_I$, max. 8 biv.	0—25
groenlandica (Hudson Bay, Canada) × *multiflora* (Styria 6, Austria)	11		0—25
» (» » ») × » (» 5, »)	15		0—25
multiflora (Caledonia Co., Vt., U. S. A.) × *vallesiaca* (Tyrol 7, Austria)	24	chromosome associations +univalents: 17—23	sterile
vallesiaca (Tyrol 2, Austria) × *multiflora* (Caledonia Co., Vt., U. S. A.)	0		
» (Tyrol 5, Austria) × *multiflora* (Mt. Washington, N. H., U. S. A.)	11	degenerated	sterile
multiflora (Styria 7, Austria) × *vallesiaca* (Tyrol 5, Austria)	28	chromosome associations +univalents: 10—13	50—60
» (Massif Central, France) × *vallesiaca* (Tyrol 13, Austria)	30		0—25
vallesiaca (Tyrol 5, Austria) × *multiflora* (Styria 6, Austria)	21	chromosome associations +univalents: 14—20 (Fig. 5 *e*)	70—80
» (» » ») × » (» 7, »)	15	chromosome associations +univalents: 10—12 (Fig. 5 *b*, *g*, *h*)	70—80
multiflora (Caledonia Co., Vt., U. S. A.) × *t. pallescens* (Härjedalen, Sweden)	5	usually $8_{II}+8_I$, max. 9 biv.	0—10
t. pallescens (Härjedalen, Sweden) × *multiflora* (Mt. Washington, N. H., U. S. A.)	9		0—25
t. pallescens (Härjedalen, Sweden) × *multiflora* (Styria 5, Austria)	28		70—80

generations in the cross combinations of the two geographical races of America with other tetraploids of the campestris-multiflora complex.

Second hybrid generation, F_2							
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants
		0—25	26—50	51—75	76—100		
86	67	46	23	23	8	13	5
100	(45)	(50)	(25)	(25)	—	4	—
93	74	60	31	9	—	32	7
(50)	(0)						
no progeny studied							
(83)	(80)	(50)	(50)	—	—	4	—
64	60	45	33	22	—	18	8
78	54	44	19	25	12	16	2
86	65	63	37	—	—	19	—
95	49	41	18	23	18	22	3
84	67	(63)	(25)	(12)	—	8	3
97	67	28	24	24	24	24	1
97	74	30	22	35	13	37	4
75	50	(40)	(60)	—	—	5	2
89	70						
97	72	10	45	25	20	20	1

multiflora and *groenlandica* are completely sterile with early degenerated pollen mother cells. The cross between the European *multiflora* and *groenlandica* gives, on the other hand, an F_1 hybrid with a relatively high degree of pairing of chromosomes at first metaphase and a certain fruit-setting ability. The F_2 plants have rather a high mortality, and the fertility of the F_3 progeny is low with only a few fertile plants. These facts are somewhat astonishing as the geographical distance between the original localities of these parental species differs distinctly, the one between James Bay in Northern Canada and New England being much shorter than the one between Northern Canada and the Alps in Europe. Yet, the European race of the tetraploid *multiflora* provides, as pointed out above, a more fertile hybrid with this North Canadian species than does *multiflora* of Eastern North America. A distinct difference of fertility is also demonstrated when the two *multiflora* races are crossed with the only endonuclear polyploid of the tetraploid level used in the crosses, the 36-chromosomal *vallesiaca*. When *vallesiaca* is crossed with the European *multiflora*, especially that of the Alps, a fairly fertile F_1 hybrid is obtained with a comparatively good fertility of F_2 plants; and as meiosis is not degenerated, it is, thus, possible to study. In one of the strain combinations investigated it is easy to find the 12 large associations of AL bivalent size together with 2 to 8 small univalents of BL size (Fig. 5 *e*). In the two other strain combinations studied, the pairing of chromosomes is stronger during first metaphase. Among 22 cells studied at first metaphase in polar view in one of the crosses, 7 are found to show 12 chromosome bodies of AL bivalent size (Fig. 5 *g*), but in 11 cells only 11 chromosome associations are found, 10 of which are of AL bivalent size, the last one being larger and interpreted as a »quadrivalent-association» (Fig. 5 *h*). In four cells, 8 associations of AL bivalent size together with two big associations of AL quadrivalent size have been counted. When *vallesiaca* is crossed with the North American *multiflora* a completely sterile F_1 hybrid is obtained, with a fairly low degree of pairing during first metaphase of meiosis. From the cross between the European *multiflora* and *vallesiaca* an F_2 progeny has been raised. In this third generation the chromosome numbers of the plants were investigated, in order to find out whether the chromosome numbers could be stabilized intermediately. Some of the F_2 plants studied possessed really chromosome patterns, indicating a stabilization which could be compared with the half-completed endonuclear polyploids found in nature. Thus, for instance, plants with $2n=28$ (20 AL+8 BL), $2n=26$ (22 AL+4 BL), or $2n=24$ (24 AL) were found in the F_2 progeny

together with other plants with intermediate chromosome numbers. It, therefore, seems possible to get a stabilization of the chromosome patterns in later generations of this cross between the European race of *multiflora* and *vallesiaca*. In this way the *multiflora* strain of Gross Glockner with $2n=28$ (20 AL+8 BL) might have arisen (Fig. 1 *e—f*). Finally, the results of the crosses with the artificially-made tetraploid *pallescens* are reported. In the F_1, the hybrid with the European *multiflora* is much more fertile than the one with the East American race. The F_2 progeny, originating from the cross with the European *multiflora*, are relatively fertile with 20 per cent fertile plants. In the F_2s, originating from the cross with the East American race of *multiflora*, one strain combination is almost sterile, but the other one was distinctly more fertile although the plants were accidently destroyed before full maturity.

The last Table (13) of the tetraploids presents the results of the crosses with the West American *comosa* and *groenlandica* of Arctic North America. In the preceding Tables (11—12) it is shown that *comosa* only with difficulty crosses with the tetraploid species of the *campestris-multiflora* complex discussed in these Tables. With the Japanese race of *multiflora*, no hybrids were obtained; and the few hybrids produced with the East American race are almost sterile, giving no adult plants in the second generation, whereas the hybrids with the European race are somewhat more easily obtained and a little more fertile. In Table 13 it is seen that the cross between *comosa* and *groenlandica* did not succeed, thus giving the same result as the cross with the Japanese race of *multiflora*. The hybrid between *comosa* and *vallesiaca* is rather easily obtained in one strain combination, but gives a completely sterile F_1, whereas the combination between *comosa* and the artificially-made tetraploid *pallescens* succeeds with difficulty, giving rise to an F_1 hybrid of reduced fertility, which produced an F_2 with a low viability and only a few adult sterile plants. *Groenlandica* is throughout more fertile than *comosa* in the combinations with the other tetraploids. In the preceding Tables (11—12) it is demonstrated that *groenlandica's* hybrid with the Japanese and European race of *multiflora* is comparatively fertile. In the cross with the East American race of *multiflora*, the hybrids were easily obtained but completely sterile. Table 13 demonstrates that the hybrid between *groenlandica* and *vallesiaca* is easily produced but comparatively sterile. At first metaphase only a weak pairing of chromosomes is obtained (Fig. 5 *i*), but the cells show no signs of degeneration. The combination between *groenlandica*

TABLE 13. *Results of the investigations of the first and second hybrid and the Arctic North American groenlandica (2n=24) with*

First hybrid generation, F_1				
Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent.	Germina Per ce
comosa (Vancouver Isl., Canada) × *comosa* (Tuolumne Co. 5, Calif.)	20		90–100	98
» (Tuolumne Co. 5, Calif.) × *groenlandica* (Hudson Bay, Canada)	0			
» (» » 7, ») × » (» » »)	0			
groenlandica (Hudson Bay, Canada) × *comosa* (Tuolumne Co. 3, Calif.)	0			
» (» » ») × » (» » 5, »)	0			
comosa (Tuolumne Co. 7, Calif.) × *vallesiaca* (Tyrol 5, Austria)	0			
» (» » 5, ») × » (» » »)	0			
vallesiaca (Tyrol 7, Austria) × *comosa* (Vancouver, Canada)	6		sterile	
» (» 13, ») × » (Tuolumne Co. 7, Calif.) ...	0			
comosa (Tuolumne Co. 5, Calif.) × *t. pallescens* (Härjedalen, Sweden)	0			
t. pallescens (Härjedalen, Sweden) × *comosa* (Tuolumne Co. 5, Calif.)	1	usually $6_{II}+12_I$	0–25	29
groenlandica (Hudson Bay, Canada) × *vallesiaca* (Tyrol 7, Austria)	6	degenerated	sterile	
» (» » ») × » (» 13, »)	13	»	0–10	80
vallesiaca (Tyrol 5, Austria) × *groenlandica* (Hudson Bay, Canada)	22	weak pairing (Fig. 5 *i*)		
» (» 13, ») × » (» » »)	14		0–10	87
groenlandica (Hudson Bay, Canada) × *t. pallescens* (Härjedalen, Sweden)	14		25–50	81
t. pallescens (Härjedalen, Sweden) × *groenlandica* (Hudson Bay, Canada)	3	usually $6_{II}+12_I$	25–50	no
t. pallescens (Härjedalen, Sweden) × *vallesiaca* (Tyrol 6, Austria) ...	7	multi-associations + univalents: 14–19	70–80	78
vallesiaca (Tyrol 4, Austria) × *t. pallescens* (Härjedalen, Sweden) ...	3			
» (» 5, ») × » (» ») ...	4		70–80	90

and the tetraploid *pallescens* is somewhat more fertile in both first and second generation than the crosses between *groenlandica* and *vallesiaca*.

Finally, the combination between the endonuclear polyploid of the Alps, *vallesiaca* (2*n*=36), and the artificially-made tetraploid *pallescens*, is shown in the Table (13). This hybrid is rather fertile in both first and second generations. It has given about the same fertility as the combination between *vallesiaca* and the European *multiflora*.

generations in the crosses between the West American comosa (2n=24) the other tetraploids of the campestris-multiflora complex.

Fruit-setting ability. Per cent.	Second hybrid generation, F_2							
	Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants
			0—25	26—50	51—75	76—100		
90—100	98	71	18	—	14	68	34	5
sterile								
0—25	29	(71)	(67)	(33)	—	—	3	—
sterile 0—10	80	81	(86)	(14)	—	—	7	4
0—10	87	(62)	(67)	(33)	—	—	6	2
25—50	81	55	(45)	(45)	(10)	—	9	8
25—50	no progeny studied							
70—80	78	74	19	19	39	23	31	4
70—80	90	67	53	30	17	—	30	9

HYBRIDS BETWEEN TWO GEOGRAPHICAL RACES OF THE HEXAPLOID L. MULTIFLORA

All taxonomical units of the *campestris-multiflora* complex belonging to the hexaploid level of polyploidy are referred to the collective species *L. multiflora*. Such hexaploids have been studied from Europe and Eastern North America. They also occur in Greenland (BÖCHER, 1950); but no material from those regions has been used in the crosses. In

TABLE 14. *Results of the crosses between*

First hybrid generation, F_1			
Species and original localities of parents	No. of F_1 plants obtained	Meiosis	Fruit-setting ability. Per cent
multiflora (Ottawa, Ont., Canada) × *multiflora* (Sweden)	1	degenerated	
» (Cypress Hills, Man., Canada) × *multiflora* (Sweden)	8	»	sterile
» (Medelpad, Sweden) × *multiflora* (Mt. Washington, N. H., U. S. A.)	3	16—18 biv., partially degenerated	25—50
multiflora (Medelpad, Sweden) × *multiflora* (Mt. Washington, N. H., U. S. A.)	9	partially degenerated	25—50
frigida (Härjedalen, Sweden) × *multiflora* (Mt. Washington, N. H., U. S. A.)	2		0—10
frigida (Härjedalen, Sweden) × *multiflora* (Cypress Hills, Man., Canada)	4	partially degenerated	0—25
multiflora (Mt. Washington, N. H., U. S. A.) × *frigida* (Härjedalen, Sweden)	3	degenerated	sterile
multiflora (Cypress Hills, Man., Canada) × *frigida* (Härjedalen, Sweden)	10	»	0—25
multiflora (Massif Central, France) × *multiflora* (Silkeborg, Denmark)			90—100
multiflora (Silkeborg, Denmark) × *multiflora* (Massif Central, France)		18_{II}	90—100
frigida (Härjedalen, Sweden) × *multiflora* (Medelpad, Sweden)	20	18_{II}	90—100
multiflora (Medelpad, Sweden) × *frigida* (Härjedalen, Sweden)	10 ±		90—100

Scandinavia we have a mountain race of this hexaploid species, which has been described under the name of *L. frigida sensu* SAMUELSSON (LINDMAN, 1926). In the Alps in Europe the alpine ecotype of *L. multiflora* belongs to the tetraploid level of polyploidy, on account of which this race of *multiflora* more nearly resembles *L. sudetica* of the diploid level than is the case with the hexaploid *frigida* of the Scandinavian mountains. In the Tables the alpine ecotype of the hexaploid *multiflora* of the Scandinavian mountains is named »*frigida*».

The results of the crosses between the hexaploid races of *multiflora* are demonstrated in Table 14. Here it is shown that the crosses between all the different taxonomical units of the hexaploid *multiflora* easily gave results. The fertility of the F_1 hybrids is, however, very different. In some combinations, as the one between a strain of Cypress Hills, Manitoba, and a Swedish one, the hybrid is completely sterile. The same is true of the combination between *multiflora* of Mt. Washington in

geographical races of the hexaploid L. multiflora.

Second hybrid generation, F_2							
Germination. Per cent	Viability of F_2 plants. Per cent	Fruit-setting ability of F_2 plants. Per cent				No. of F_2 plants investig.	No. of non-flowering F_2 plants
		0—25	26—50	51—75	76—100		
68	79	47	27	13	13	15	—
86	95	36	31	17	16	55	18
36	(60)	(33)	(67)	—	—	3	—
60	(33)	—	—	—	—	—	2
(46)	(50)	(50)	(50)	—	—	2	1
97	76	26	11	—	63	19	3
97	93	—	11	—	89	27	
97	94	—	—	—	100	56	1
no progeny studied							

New Hampshire and the Scandinavian *frigida*. Moreover, no hybrids between Europe and North America have more than 50 per cent fruit-setting ability. At first metaphase the F_1 hybrids between the European and the North American material usually showed signs of degeneration. The only strain combination possible to study, however, formed 16 to 18 bivalents.

In the second hybrid generation the viability and fertility of the plants are very variable, depending on the origin of the parental strains used in the crosses. When the European and North American races are crossed, no or a few very sterile F_2 plants are obtained, with one exception only. This exception is the combination between a Swedish *multiflora* and two strains collected in the vicinity of each other near the foot of Mt. Washington in New Hampshire. In this combination the viability of the F_2 progeny is comparatively good and a few fertile F_2 plants are obtained.

When hybrids are made between different races of the hexaploid *multiflora*, originating from Europe, all the F_1 plants are fertile as well as those possessing the Scandinavian alpine ecotype *L. frigida sensu* SAMUELSSON as one of their parents. At first metaphase the hybrids investigated had formed 18 bivalents. The second hybrid generation has a god viability and the majority of the F_2 plants are fertile. The strain combinations between Massif Central, France, and Denmark, however, give some sterile plants in the F_2. The cross between *frigida* and the Swedish *multiflora* is, however, fully fertile in both the first and second generations.

HYBRIDS BETWEEN DIFFERENT LEVELS OF POLYPLOIDY

Hybridization between different levels of polyploidy has also been tried in the investigation of the *campestris-multiflora* complex. Table 15 demonstrates the compatibility between the different levels of polyploidy. Species belonging to the diploid level are not possible to hybridize with species belonging to any other level of polyploidy. Hybridization attempts have been made with species of the tetraploid, hexaploid and octoploid level of polyploidy, but with no success. Species of the tetraploid level of polyploidy are easily crossed with species of the hexaploid level. F_1 hybrids obtained are fairly fertile giving an offspring with somewhat varying chromosome numbers (Table 16). Species of the tetraploid level are, however, not possible to hybridize with octoploids. Hybridization between these two levels of polyploidy has been tried on rather a large scale in order to investigate whether it would be

TABLE 15. *Compatibility between species of different levels of polyploidy of the campestris-multiflora complex.*

(×=compatible combination; —=incompatible combination.)

Level of polyploidy	Diploid	Tetraploid	Hexaploid	Octoploid
Diploid	×	—	—	—
Tetraploid	—	×	×	—
Hexaploid	—	×	×	×
Octoploid	—	—	×	×

TABLE 16. *Chromosome numbers found in the progeny of the hybrids between tetraploid and hexaploid L. multiflora.*

$2n$:	29	30	31	32	33	34
Frequency:	1	9	3	—	—	1

possible to produce hexaploids in such a way. But these attempts failed entirely. Thus, there is no reason to believe that the hexaploids arose in such a manner. Finally, the species of the hexaploid level of polyploidy are easily crossed with those of the octoploid level. The hybrids are fairly fertile and give rise to a progeny with varying chromosome numbers.

Compatibility between the endonuclear polyploids follows the level of polyploidy and not the number of chromosomes. Thus, *sudetica* ($2n=48$) of the diploid level easily crosses with the 12-chromosomal diploids (pp. 33—37) but does not give any hybrids with *congesta* ($2n=48$) of the octoploid level of polyploidy.

NATURALLY OCCURRING HYBRIDS BETWEEN SPECIES OR CHROMOSOMAL RACES OF THE CAMPESTRIS-MULTIFLORA COMPLEX

Naturally occurring hybrids between species of the diploid level of polyploidy have been studied in Sweden. In Scandinavia we have three species of the *campestris-multiflora* complex belonging to the diploid level. These are *L. campestris s. str.* ($2n=12$), *L. pallescens* ($2n=12$), and *L. sudetica* ($2n=48$). Where two of these three species occur together, hybrids are very often found. *Campestris* usually grows in dry, sunny places, but it has sometimes been found in clearings in the woods. Here, it is likely to meet *pallescens* in those areas of Scandinavia where these two species occur together. In such an opening in Kila, province of Södermanland, Sweden, a hybrid population was found. The plants were intermediate between *campestris* and *pallescens*, and thus rather similar to the collective species *L. multiflora*. The chromosome numbers were, however, $2n=12$, and a tendency to form stolons, found in some individuals, revealed the *campestris* origin. A specimen of a similar hybrid is found in the Herbarium of Upsala University. The plants were collected by G. SAMUELSSON in Stora Skedevi in the province of Dalecarlia, an area in which *campestris* as well as *pallescens* are known. In this case the hybrid specimen has been referred to *L. multiflora*, but the tendency to form stolons reveals its origin. Hybrids between *sudetica* and the two diploids *campestris* and *pallescens* are reported from many places. These hybrids are sterile and thus easy to recognize in nature. In several localities where *campestris* and *sudetica* are found together, the hybrids are common (HYLANDER, 1953). Still more frequent is the growing together of *pallescens* and *sudetica*, as both these species have

a northern distribution in Scandinavia, and natural hybrids are reported from several localities (HYLANDER, 1953). Hybrids between the polyploid *multiflora* or its alpine subspecies *frigida* and *sudetica* are also frequently reported. Such hybrids between *sudetica* of the diploid level and *multiflora* of a polyploid level have not been obtained experimentally. Reported hybrids of this kind are thus likely to be the hybrid between *pallescens* and *sudetica*. Specimens indicated as hybrids between *multiflora* and *sudetica* have not been possible to distinguish from the ones determined as *pallescens*×*sudetica*. Moreover, in almost all localities where the former hybrid has been reported, a statement of the occurrence of *pallescens* has also been given. In a study of the *campestris-multiflora* complex of the genus *Luzula* in Switzerland, SAMUELSSON (1922) reports that the hybrid between *multiflora* and *sudetica* does not occur in this part of Europe. *Sudetica* is found in the Alps and so this is also the case with the tetraploid and hexaploid *multiflora*. The diploid *pallescens* is, however, found only in the eastern parts of the Alps, and the most western locality from which I have received material of this species is Kufstein in Tyrol; from Switzerland, however, it is not reported. It is therefore not astonishing that no sterile hybrids with *sudetica* are found in the region of western Switzerland, where SAMUELSSON made his study. From these facts it may be concluded that it is not very likely that any hybrids between a polyploid *multiflora* and *sudetica* occur in nature.

TABLE 17. *Chromosome numbers found in two hybrid swarms collected in Jutland in Denmark.* (*Locality A is situated 13 km North of Skern and locality B at Gelsaa between Gram and Ribe.*)

$2n$:	36	37	38	39	40	41	42	43	44	45	46	47	48
Frequency at A:	5	—	—	1	—	—	2	—	2	—	—	1	3
» » B:	1	—	—	1	1	—	6	—	—	—	—	—	4

In regions where tetraploid and hexaploid *multiflora* are found together, hybrids between the two races occur. In Bretton Woods, N. H., near the foot of Mt. Washington in New England, the two races of *multiflora* and their hybrids were found together in one collection. Thus, a continuous variation from the more slender tetraploid type to the coarser hexaploid is found; and a determination of a specimen according to its level of polyploidy is then almost impossible with material collected in nature. The chromosome numbers of the progeny of such natural hybrids with $2n=30$ are given in Table 16. The chromosome numbers range from 29 to 34, and the majority of the plants have $2n=30$.

Where the hexaploid *multiflora* occurs together with the octoploid *congesta*, hybrid swarms are found. This fact makes it very difficult to distinguish the true *congesta* from *multiflora* in such localities. The chromosome numbers determined on plants from two such localities in western Jutland, Denmark, are given in Table 17.

DISCUSSION AND CONCLUSIONS

To obtain an expression of the different degree of differentiation between the taxonomical units of the *campestris-multiflora* complex involved in the crossings, diagrams showing the relationship between these taxonomical units have been worked out from the figures of the Tables. In the diagrams the thickness of the rods demonstrates the fertility of the F_1 hybrids and the number of the thin black lines gives a measure of the fertility of the second hybrid generation. Those giving more than 75 per cent fruit-setting ability have been referred to the fertile plants of the F_2 progeny, thus corresponding to the figures of the last column of fertility of F_2 plants in the Tables. When the crosses between different strains of the same species have given deviating results, then the arithmetical mean has been taken as a measure. Only in a few combinations, however, are the results of the crosses really distinctly dissimilar. This is the case in the strain combinations with the East American species *echinata*. It seems as if the strain originating from Morris Co., N. J., gives distinctly more fertile hybrids in all different combinations, when it is used, than the other two strains of that species, in spite of the fact that the original localities of the three strains are not situated very widely apart (Moris Co., New Jersey, Philadelphia, Penn., and Washington, D. C.).

As the first diagram demonstrates (Fig. 6), the two diploids of Japan, *lutescens* and *capitata*, are closely related to each other and to *pallescens* of Europe as well as to *bulbosa* of Eastern North America, in spite of the fact that *bulbosa*, especially, originates from a locality far removed from Japan. *Campestris* and *echinata* are less related to the Japanese species and this is also the case with all the West American species. When we have Europe in the centre of the diagram (Fig. 7), we find that *pallescens* usually is more closely related to all the other diploids than is the case with *campestris*. *Echinata* is, however, an exception, giving more fertile hybrids with *campestris* than with *pallescens*. The affinity between *campestris* and *pallescens* is relatively great, as seems usually to be the case between species originating from the same

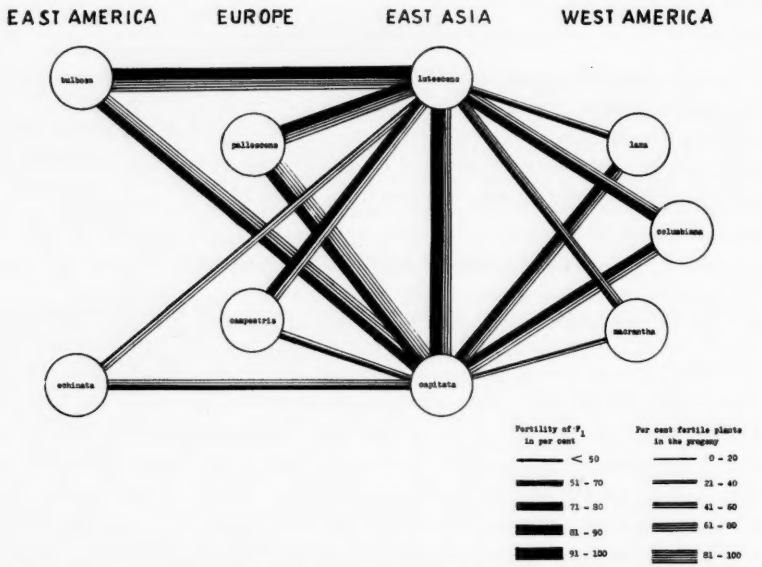


Fig. 6. Diagram demonstrating the fertility of the first and second hybrid generations in the cross combinations between the two Japanese diploids and the other diploid species of the *campestris-multiflora* complex.

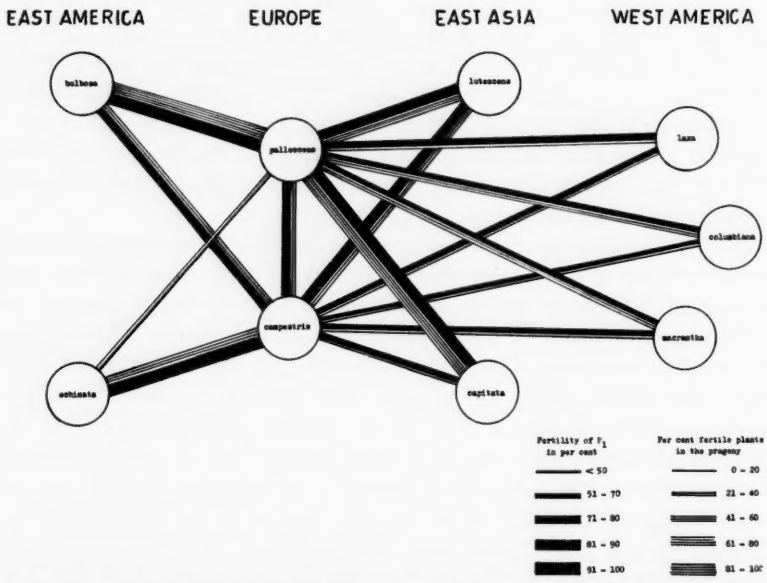


Fig. 7. Diagram demonstrating the fertility of the first and second hybrid generations in the cross combinations between the two European diploids and the other diploid species of the *campestris-multiflora* complex.

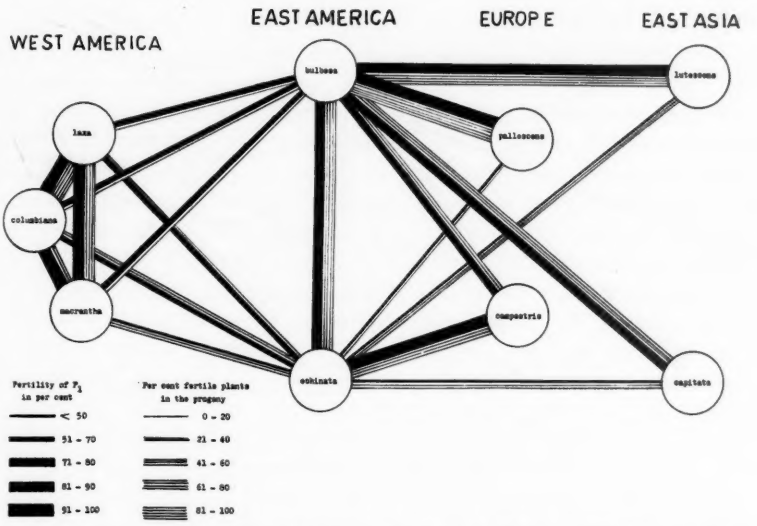


Fig. 8. Diagram demonstrating the fertility of the first and second hybrid generations in the cross combinations between the two East American diploids and the other diploid species of the *campestris-multiflora* complex.

geographical area, but not as great as the affinity between *pallescens* and *bulbosa* of Eastern North America or *lutescens* and *capitata* of Japan. The hybrids between *pallescens* and *bulbosa* have a full fertility in both first and second generations. The F_1 hybrid between these two species is very difficult to distinguish from its parents. A reliable method for doing this is to study whether segregation occurs in F_2. These two species or varieties seem to be the only ones of all the diploids of the complex studied here which really do not show any sterility barrier between them. Thus, they ought to be referred to the same species in spite of the fact that they originate from different geographical areas. On the other hand, *echinata* shows a distinctly different affinity to *bulbosa*, compared with that to *pallescens* (Fig. 8).

The third diagram for the diploids (Fig. 8) shows the East American species in the centre. Here we find again the close relation between *bulbosa* and the Japanese species as well as *pallescens* of Europe, in contrast to the stronger differentiation revealed between the two East and the three West American species. The two species of the East American region are closely related, as is usually the case with ecologically separated species of one and the same area.

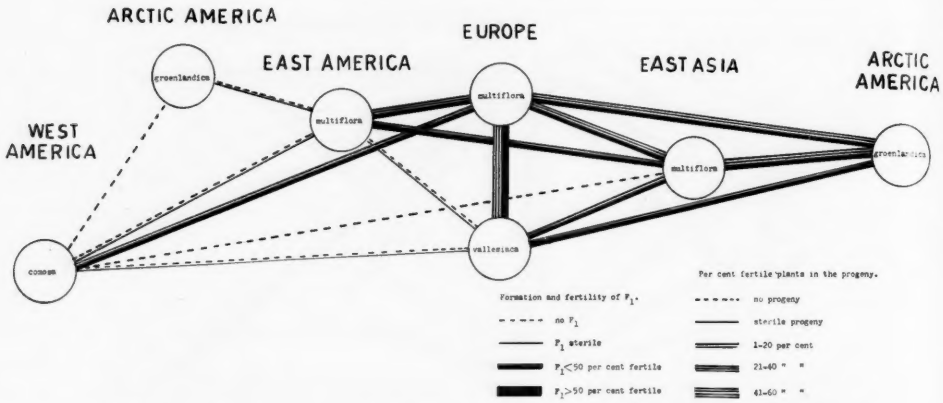


Fig. 9. Diagram demonstrating the fertility of the first and second hybrid generations in the crosses between different taxonomical units of the tetraploid level of polyploidy of the *campestris-multiflora* complex.

The species of the West American region are all closely related to each other (Fig. 8). This is not only the case with the two *comosa* varieties, *macrantha* and *laxa*, but *columbiana* also behaves in the same way, in spite of the fact that it morphologically resembles *multiflora* more than *comosa*.

The last diagram (Fig. 9) demonstrates the affinity between the taxonomical units of the tetraploid level of polyploidy. This diagram shows a larger variability in differentiation between different taxonomical units than do the diploids. On the other hand, the diagram follows the same general pattern, and this is especially true of the West American species, *comosa*, which has reached the strongest differentiation of all species used in the crosses. Of great interest is the North Canadian species, *groenlandica*, which seems to be more related to the Japanese and European races of *multiflora* than to the East American one, in spite of the fact that the latter geographically is situated much closer to *groenlandica* than the former.

When surveying the results of the crosses of the *campestris-multiflora* complex investigated here there are certain general facts applicable to the taxonomical units of all the different levels of polyploidy which may be pointed out. Firstly, we are faced with the phenomenon that species and varieties belonging to the same geographical regions but ecologically separated usually have a fairly strong genetical affinity to each other,

an affinity which, as a rule, is stronger than that of the geographically separated taxonomical units. Among the species of the diploid level of polyploidy we have usually tow, or in Western North America three, 12-chromosomal species of each geographical region. These species generally give a rather fertile F_1 hybrid and no degeneration of pollen mother cells has been found in such combinations. The fertility of the second generation is usually very variable ranging from more or less reduced to full fertility; but very often a rather high percentage of fertile plants come out. This is the case with the combinations between *lutescens* and *capitata* of Japan, *bulbosa* and *echinata* of Eastern North America, and *pallescens* and *campestris* of Europe as well as those between the three West American species *macrantha*, *laxa*, and *columbiana*. This same phenomenon of a great affinity between species of the same geographical area holds also true for the endonuclear polyploids. We have, for instance, *sudetica* of Europe with 48 small CL chromosomes, a species which easily crosses with all the 12-chromosomal diploids. Here the F_1 hybrids are sterile, something that is easily understandable if we take into account mechanical disturbances during chromosome separation at meiosis. But if meiosis of the hybrids is studied, it is easy to find differences between the different hybrid combinations. Meiosis of the F_1 hybrid between *sudetica* and the European diploids *pallescens* or *campestris* is possible to study, even though difficulties may arise on account of the very small chromosomes (see p. 33). The F_1 hybrids together with other species of the diploid level of polyploidy as, for instance, *echinata*, *laxa*, *macrantha*, or *orestra* were easy to produce (Tables 9—10), but meiosis was impossible to study as the pollen mother cells were more or less degenerated at an early stage. A corresponding result was obtained in the crosses between *orestra* ($2n=22$) of Western North America and other species of the diploid level of polyploidy. Meiosis of the F_1 hybrids between *orestra* and *laxa* or *macrantha* was possible to study (see p. 35), whereas that between *orestra*, on the one hand, and *capitata*, *campestris*, or *sudetica*, on the other, was more or less degenerated at first metaphase (Table 10).

Among the species of the tetraploid level of polyploidy, *vallesiaca* with $2n=36$ is the only endonuclear polyploid used in the crosses. This species can be hybridized with all other species or varieties of the tetraploid level. The only hybrid combination, however, which gives a fairly fertile F_1 hybrid and F_2 progenies with at least some fertile plants is that with the European *multiflora* from the Alps, *i. e.*, the tetraploid from the same geographical area as *vallesiaca*.

TABLE 18. *Fruit-setting ability, expressed in per cent, found for the F_1 hybrids of the most fertile strain combination of indicated species cross between diploids of the campestris-multiflora complex.*

Region occupied	Species originating from areas around the North Pole						Species originating from Western North America			Mean fertility of F_1s for indicated species
	holding the northern distribution			holding the southern distribution						
Species	*lutescens*	*pallescens*	*bulbosa*	*capitata*	*campestris*	*echinata*	*laxa*	*macrantha*	*columbiana*	
	lutescens	90—100	90—100	*90—100*	90—100	50—70	50—70	70—80*	90—100	82
		pallescens	90—100	90—100	*80—90*	50—70	80—90	90—100	50—70	84
			bulbosa	80—90	70—80	*90—100*	50—70	70—80*	50—70	78
				capitata	50—70	50—70	70—80	50—70*	90—100	76
					campestris	90—100*	50—70	50—70*	50—70*	72
						echinata	80—90*	50—70*	50—70	69
							laxa	*90—100*	*90—100*	71
								macrantha	*90—100*	71
									columbiana	72

* Abnormal F_1 plants or hybrid seeds did not germinate in one or two strain combinations.

TABLE 19. *Percentage of fertile plants in F_2 found in the most fertile strain combination of indicated species cross between diploids of the campestris-multiflora complex.*

Region occupied	Species originating from areas around the North Pole						Species originating from Western North America			Mean percentage for indicated species
	holding the northern distribution			holding the southern distribution						
Species	*lutescens*	*pallescens*	*bulbosa*	*capitata*	*campestris*	*echinata*	*laxa*	*macrantha*	*columbiana*	
lutescens	92	79	44	25	30	11	37	25	43	
pallescens	100	72	60	13	21	48	23	53		
bulbosa	43	22	76	14	22	3	40			
capitata	16	31	29	37	35	38				
campestris	42	0	6	3	16					
echinata	37	55	31	34						
laxa	91	88	19							
macrantha	26	34								
columbiana	20									

Thus, the hybridization experiments between endonuclear polyploids also stress the fact that taxonomical units of the *campestris-multiflora* complex originating from one and the same geographical area usually are genetically closely related. This affinity might be interpreted as being caused by common origin or as the result of gene exchange between species of a common area. Concerning the endonuclear polyploids, it seems very likely that species of this kind must be of a comparatively young origin, at least in the *campestris-multiflora* complex. Otherwise it is difficult to explain their close relation to the true polyploids of the same geographical region, as very little gene exchange is likely to occur between species of this kind, at least between such species of the diploid level of polyploidy.

The second general piece of information which can be derived from the Tables and diagrams is the fact that certain species or varieties are found which, more easily than the rest cross with all the other species of their own level of polyploidy. Among the diploids, such species are, for instance, *pallescens* of Europe and *lutescens* of Japan, and to some extent *bulbosa* of Eastern North America. Tables 18 and 19 demonstrate these facts. The schedule of Table 18 shows the fruit-setting ability of the F_1 hybrids of the most fertile strain combinations in each species cross. The species are arranged according to their geographical origin. Placed in the three first columns are the species occupying an almost circumpolar area around the North Pole and holding the northern distribution of this area (*pallescens*, *lutescens*, and *bulbosa*); the next three columns give the species originating from the same geographical areas but holding the southern distribution (*capitata*, *campestris*, and *echinata*); and lastly the three West American species (*laxa*, *macrantha*, and *columbiana*) are presented. A mean of the fruit-setting ability of the F_1 hybrids of all combinations with a certain species is given in the last column. The figures giving the fertility of the F_1 hybrids between species belonging to the same geographical area are in *italics*, and not taken into consideration when the means are calculated. The exclusion of the combinations mentioned is due to the fact that these hybrids are usually more fertile than the others and, thus, they will change the means according to the number of taxonomical units distinguished in, or obtained for investigation from a certain geographical area. In the schedule we find that the species occupying the circumpolar regions around the North Pole, *i. e.*, *lutescens* of Japan, *pallescens* of Europe, and *bulbosa* of Eastern North America, are those giving the most fertile hybrids with each other. The means given for these species are also the

highest, compared with the means given for the other species. The same high fertility also appears in the second hybrid generation (Table 19). The highest mean fertility in both first and second generations for all the diploid taxonomical units tested is obtained for *pallescens*. The three species occupying regions south of the circumpolar area, *i. e.*, *capitata* of Japan, *campestris* of Europe, and *echinata* of Eastern North America, usually have a lower fertility than the three first-mentioned species, when crossed with other taxonomical units of the complex (see last columns of Tables 18 and 19). The same low fertility in both first and second generation is also demonstrated for the three West American species when they are crossed with species originating from the other geographical regions (Tables 18 and 19). When the West American species are crossed among each other the hybrids have a comparatively high fertility.

In the schedule of Table 18 the occurrence of different types of abnormal F_1 hybrids is stated with an asterisk for the species combinations where they have occurred. All such abnormalities, with one exception only, occur in combinations where one West American species is involved, and the majority of these combinations are in hybrids with *macrantha* or *campestris*, and at least one of the species involved always seems to originate from a dry habitat.

Among the tetraploids it is only the artificially-made tetraploid *pallescens* which in general gives fertile F_1 hybrids in combination with other tetraploids. The fertility of the combinations between the tetraploid taxonomical units is demonstrated in Tables 20 and 21. In the Tables the tetraploid species and geographical races of *multiflora* are arranged according to their order around the North Pole, and the artificially-made tetraploid *pallescens* is placed in the last column. The figures in Table 20 indicate the highest fertility of the F_1 hybrid obtained in the combinations in question. In the schedule it is easily found that the only fertile combination is the one between the two European species, in spite of the fact that they have different chromosome numbers. All the other combinations are between species and races originating from different geographical areas. The mean fertility for the combinations with other tetraploid taxonomical units is highest for the *multiflora* race of Japan and that of Europe. *Groenlandica* of Arctic North America and *multiflora* of Eastern North America show about the same rather low affinity to the other tetraploids but are sterile when tested against each other; and *comosa* is the tetraploid species which demonstrates the lowest affinity to all the other species of the same level

TABLE 20. *Fruit-setting ability, expressed in per cent, found for the F_1 hybrids of the most fertile strain combination of indicated cross between the tetraploid taxonomical units of the campestris-multiflora complex.*

Arctic North America	Japan	Europe		E. North America	W. North America	Mean fertility of F_1s for indicated species	Fertility of F_1 between *t. pallescens* and indicated species
groenlandica	*multiflora*	*multiflora*	*vallesiaca*	*multiflora*	*comosa*		
groenlandica	0—25	0—25	0—10	0	0	6	25—50
	multiflora	25—50	0—10	0—25	—	14	70—80
		multiflora	*70—80*	0—25	0—10	17	70—80
			vallesiaca	0	0	3	70—80
				multiflora	0—10	6	0—25
					comosa	2	0—25

TABLE 21. *Percentage of fertile plants in F_2 found in the most fertile strain combination of indicated species cross between tetraploid taxonomical units of the campestris-multiflora complex.*

Arctic North America	Japan	Europe		E. North America	W. North America	Mean percentage of indicated species	Percentage of fertile F_2 plants in crosses between *t. pallescens* and indicated species
groenlandica	*multiflora*	*multiflora*	*vallesiaca*	*multiflora*	*comosa*		
groenlandica	25	12	0	—	—	7	0
	multiflora	17	0	0	—	8	51
		multiflora	24	8	0	9	20
			vallesiaca	—	—	0	23
				multiflora	0	2	0
					comosa	0	0

of polyploidy. The last column of Table 20 gives the fertility of the F_1 hybrids with the artificially-made tetraploid *pallescens*. Here, the fertility is throughout much higher than for the crosses between the natural taxonomical units.

Table 21 demonstrates the fertility of the F_2 generations. This is measured by the percentage of fertile plants in the F_2 progeny. It gives about the same pattern as that of the first generation. The relatively highest average fertility is that found between the tetraploid races of *multiflora* originating from Japan and Europe as well as *groenlandica* of Arctic North America. *Multiflora* of Eastern North America gives fertile plants in the F_2 progeny only in combination with the European *multiflora*. With *groenlandica* it gives a completely sterile F_1 hybrid with no F_2 progeny. Thus, we have a relatively high fertility in both first and second generations, in the combinations between the tetraploid taxonomical units of Eastern North America and Europe, between those of Europe and Japan, and between *multiflora* of Japan and *groenlandica* of Arctic North America. In the combination between *groenlandica* of Arctic North America and *multiflora* of Eastern North America we have, however, a complete sterility, if the species are crossed with each other. Here, there seems to be a barrier between these two regions, *groenlandica* showing more affinity to the Japanese race of *multiflora* than to the East American one and the East American race of *multiflora* being more closely related to the European *multiflora* than to *groenlandica*. Also a consistently higher fertility for certain taxonomical units can be traced among the tetraploids, even though the differences between the taxonomical units of the tetraploid level are more difficult to demonstrate than those between the diploids on account of the very low fertility of the combinations between certain tetraploid taxonomical units. The generally most fertile tetraploids are, however, found in Europe and Japan.

Such species or taxonomical units which generally are easy to cross with related species have also been found in other species complexes. Species of that kind are found by GAJEWSKI (1954) in his crosses between different *Geum* species. It is *G. rivale* and to some extent *G. coccineum* which seem to give the most fertile hybrids when tested with each other and against other species of the *Eugeum* complex. A corresponding phenomenon is known to occur, for instance, in Chinese wheat, which is much more easily crossed with rye than is the case with other races of that species (BACKHOUSE, 1916).

If the results of the hybridization experiments obtained within the

different levels of polyploidy are compared with each other, certain dissimilarities between the different levels of polyploidy will emerge. The most striking of these dissimilarities is the fact that the hybridization is much easier to perform, and the fertility of both the first and second hybrid generations is much higher throughout the experiments in the species belonging to the diploid level compared with those same properties in the experimental series of the taxonomical units of the tetraploid or higher levels of polyploidy. These facts are rather astonishing, as it is usually assumed to be easier to cross polyploid species than diploids and in most cases such hybrid products are known to be relatively more fertile, if they have polyploid chromosome numbers, than when they originate from diploids. This latter fact is shown to hold true in, for instance, *Galium* (EHRENDORFER, 1954), or *Phleum* (NORDENSKIÖLD, 1945). Most astonishing are those results of the crosses within the *campestris-multiflora* complex, if the date of origin of diploids and polyploids is taken into consideration. It must be postulated that the polyploids are of a younger origin than the diploids, as the former most likely have arisen through chromosome multiplication from the latter. As the diploids still are relatively closely related and usually give fertile hybrids, we ought to expect polyploids that have arisen from those diploids to behave in the same way. This is, however, as pointed out above, not the case. A likely explanation for the further differentiation of the polyploids might be a difference in the capacity of accumulating mutations for species belonging to different levels of polyploidy. As the geographically, highly expanded pattern of distribution demonstrates, the whole *campestris-multiflora* complex of the genus *Luzula* must be of a very old origin. This fact is probably applicable to both diploids and polyploids. Thus, differences in the capacity of accumulation of mutations must result in a considerable disparity of differentiation between the taxonomical units at the present time. The polyploids ought to be the ones with the higher capacity for accumulation of mutations because of the lower selection of mutations which polyploids always have. Many mutants, lethal or sublethal for diploids, are not necessarily destructive for polyploids. In the polyploids we always find several reduplications in partly homologous chromosome sets, buffering the lethal gene of a locus. If the selection for mutations is very severe for the diploids, the polyploids might be able to accumulate mutations to such an extent that the genetical differentiation between the polyploid species would be stronger than between the diploids, in spite of their younger origin. The artificially-made tetraploid *pallescens*

had much more fertile hybrids with all the natural tetraploids than any combinations between the natural taxonomical units of the tetraploid level of polyploidy (Tables 20 and 21). This newly-made tetraploid has had no time to accumulate mutations when on the tetraploid level of polyploidy.

Another dissimilarity between the results of the hybridization experiments of the diploid and polyploid levels is the regular occurrence of white seedlings in many of the F_2 generations of the diploids. Among the polyploids only two white seedlings have been observed, one among the tetraploids and one among the hexaploids. This regular occurrence of lethal segregates among the species of the diploid level of polyploidy indicates that these species must be regarded as true diploids and that the basic chromosome number for the species of the *campestris-multiflora* complex is $x=6$. This assumption must be made in spite of the fact that the species *L. purpurea* has $2n=6$.

This study of the compatibility between the different geographical races of the *campestris-multiflora* complex and the properties of their hybrids has been made in an attempt to obtain information about the affinity between the different taxonomical units, and to obtain experimental data concerning these phenomena. From the data obtained it might be possible to get information about the evolution of the taxonomical units of the complex. It has been postulated that the degree of incompatibility and hybrid sterility is a measure of the physiological differentiation between the taxonomical units tested; species, which are easily crossed and give fertile hybrids, are, thus, less differentiated than the ones giving less fertile hybrids. If it, moreover, is postulated that species which in general give more or less fertile hybrids with other taxonomical units of a complex are less differentiated than the ones generally giving sterile hybrids, it must be assumed that species holding the first mentioned characteristics are of a more primitive origin than the last-mentioned ones. On this account the diploid species of the *campestris-multiflora* complex, occupying the circumpolar region around the North Pole and holding the northern distribution, *i. e.*, *pallescens*, *lutescens*, and *bulbosa*, must be closely related and the ones with the lowest degree of differentiation among the taxonomical units of the complex. The circumpolar diploid species holding the more southern distribution, *i. e.*, *campestris* of Europe, *capitata* of Japan, and *echinata* of Eastern North America, are more differentiated than those holding the northern distribution. On the other hand, the former are usually most closely related to the species occupying the same geographical

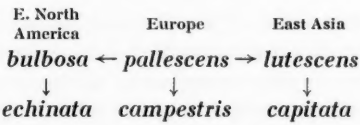


Fig. 10. Schedule of a likely evolution of the diploid taxonomical units of the *campestris-multiflora* complex.

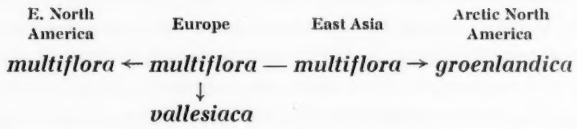


Fig. 11. Schedule of a likely evolution of the tetraploid taxonomical units of the *campestris-multiflora* complex.

region, wherefore it is justifiable to assume that they must be derived from the species holding the northern distribution of their area (Fig. 10). The West American species, both diploids and tetraploids, have probably been separated and differentiated at an early stage. The highest affinity between the West American species and other taxonomical units of the complex seems to be shown by *pallescens*, *lutescens*, and to some extent by *capitata*.

The tetraploids have in general a more southern distribution than the northern circumpolar diploids, such as *pallescens* and *bulbosa*, the distributions of which are fairly well known. The only tetraploid with a northern distribution is *groenlandica* and in the area of that species no diploid is known. The geographical races with the most fertile hybrids in combination with the other tetraploids are *multiflora* of Europe and that of Japan. Thus, they ought to be the least differentiated ones. *Groenlandica* is comparatively fertile in combinations with the Japanese and European *multiflora*, whereas *multiflora* of Eastern North America is more related to *multiflora* of Europe and to that of Japan than to *groenlandica*. These facts indicate that Eurasia might be the area from which the tetraploids have been distributed eastwards to Northern Canada and westwards to Eastern North America (Fig. 11). The tetraploid *pallescens* of Europe shows the same general pattern as the Eurasian *multiflora*, even though it gives much more fertile hybrids. It is most fertile with *multiflora* of Europe and that of Japan and less fertile with *groenlandica*, *multiflora* of Eastern North America or *comosa* of Western North America.

Another alternative explanation for the origin of the polyploids is, of

course, that they have arisen from diploids in their own geographical regions. If that were the case it would, however, be difficult to explain the geographically successive relationship of the tetraploids as well as the further differentiation between their taxonomical units compared with that of the diploid species originating from corresponding geographical areas.

The separation of the complex into a *multiflora* and a *campestris* group has not been possible. These two names seem to be built essentially on ecological race differences. Species or races of dry, sunny places have big dark flowers, long anthers with comparatively short filaments and large, rounded seeds compared with the taxonomical units originating from the more moist or cooler habitats. The first-mentioned characters hold good for *L. campestris s. str.* as well as the other species or races requiring a warm and sunny habitat, like *capitata* and *macrantha* or ecotypes of *multiflora* growing in such dry and warm localities. For instance, many strains of the last-mentioned species originating from the Island of Gotland in the Baltic are very similar to *campestris*. The characters of *L. multiflora* do not only characterize *L. multiflora s. str.*, but also to some extent the diploids of the moist and very often relatively cool habitats, like *pallescens*, *bulbosa*, *lutescens*, or *columbiana* as well as the octoploid *congesta*. This last-mentioned group of species has usually rather small flowers, short anthers with a relatively long filament as well as usually small, rather slender seeds. These characters are exaggerated in, for instance, *pallescens* and *bulbosa*, where both the diploidy and ecology of the habitat work in the same direction. Another ecological species goes under the name *L. frigida* BUCHENAU (1898). It is composed of the polyploid alpine type of the complex. It is described as coming from Labrador in Northern Canada and the specimen must have belonged to the species which here is referred to as *L. groenlandica* BÖCHER. But the description of BUCHENAU is followed by a report that this species (*L. frigida*) with dark flowers is also found in other alpine and arctic regions, as in the Alps of Europe and in Scandinavia. Thus, the alpine ecotypes of *multiflora* of the Alps or *L. frigida sensu* SAMUELSSON in Scandinavia as well as *L. campestris* v. *vallesiaca* would be referred to that collective species *L. frigida*, described by BUCHENAU. For that reason I have not used the name *frigida* but *groenlandica* for the tetraploid species of Arctic Canada.

All the species and varieties belonging to the *campestris-multiflora* complex of the genus *Luzula* are taxonomically very closely related. The morphologically most distinct taxonomical units of this complex belong,

however, to the diploid level of polyploidy. Among the species belonging to that level we always find several morphologically and ecologically distinct units occupying the same geographical area. Thus, the morphological differences, which always show up between taxonomical units of ecologically separated localities, constitute here the special characters. The morphological characters of these ecologically separated taxonomical units usually become distinct, as the species seldom get the opportunity to meet and to form hybrid swarms. These distinct morphological characters of the ecological species units of one and the same geographical area have made it possible for taxonomists to separate these units into species or varieties. For all diploids investigated here it has been possible to find descriptions in the literature, usually as species but sometimes as varieties of the collective species *L. campestris* or *L. multiflora*. In spite of the fact of these morphological differences between the diploid taxonomical units, they all seem to be fairly closely related genetically, and they usually form fertile hybrids with generally an almost regular meiosis.

The species of the *campestris-multiflora* complex belonging to the higher levels of polyploidy present another pattern. Almost all polyploid species of the complex have been referred to the collective species *L. multiflora*. They usually possess no distinct morphological differences between races of different geographical areas. The only geographical units which have been recognized as species is *comosa* of Western North America and *groenlandica* of Arctic North America. In all the other geographical regions the polyploids are referred to the collective species, *L. multiflora* or *L. campestris*. In each geographical area *L. multiflora* may occupy different ecological localities. In the more specific ecological habitats ecotypes have developed, but in only a few cases have they caused a splitting-up of the collective species into varieties or species. This is, however, the case with some chromosomal races, such as the Atlantic type of *multiflora*, described as *L. congesta* THUILL. ($2n=48$), or the 36-chromosomal alpine race of the *campestris-multiflora* complex, described as *L. campestris* v. *vallesiaca* BEAUV. and here taken up as *vallesiaca*. These two chromosomal races have, thus, been considered to correspond to the two varieties described by THUILLIER and BEAUVERD, respectively, in spite of the fact that in many cases they are very difficult to distinguish from the collective species *L. multiflora*. That no or very little splitting-up into species or varieties has been possible in the polyploid species of *L. multiflora* must depend on the fact that the variation within the species is fairly con-

tinuous and that the species is not restricted to certain habitats. For the latter reason, hybridization between local races may be common, a phenomenon which is followed by a more smooth and continuous variation within the species. Even if differences in chromosome numbers are present, hybridization between the races may occur (see above pp. 52—53). Also the West American collective species, *L. comosa*, is divided into subspecies and varieties. To this species the diploid *macrantha* and *laxa* are referred. These taxonomical units are in many cases very difficult to separate morphologically from the tetraploid. Thus, we have here a collective species that consists of a tetraploid, well differentiated from the other species of its level of polyploidy and diploids, which, however, easily hybridize with the other diploids of the *campestris-multiflora* complex and in many cases give relatively fertile hybrids.

The taxonomical units which have been used in this investigation sometimes correspond to species, sometimes to varieties, and sometimes to geographical races of one and the same species. Systematic unities such as species or varieties are defined by morphological differences and distinguished on account of discontinuity. The physiological differentiation does not always follow the morphological characters. Thus, some of the geographical races, as those of *multiflora*, demonstrate distinct physiological differentiation without showing the morphological properties of a species or even a variety. Among the diploids, on the other hand, there are some taxonomical units which show very slight physiological differences. If, in such cases, the morphological characters are very similar as, for instance, with *pallescens* and *bulbosa*, there seems to be no reason to keep different species names for them. On the other hand, the morphological as well as the physiological differentiations between the taxonomical units of the complex studied gradually increase from the very slight ones between *pallescens* and *bulbosa* to rather distinct ones between *comosa* and *multiflora*. Moreover, the different degree of variation within the taxonomical units complicates the distinguishing of species in the complex. Some species, such as *echinata*, show a distinct variation within the species and the strains used have behaved differently in the same species crosses. Other taxonomical units, such as *macrantha* and *laxa*, show fairly distinct morphological differences in the southern regions of their distribution in California, but in Vancouver Island, where these two varieties meet with the tetraploid *comosa*, the variation between all the three taxonomical units is relatively continuous and hybridization between the diploids must occur.

SUMMARY

(1) Hybridization experiments between different taxonomical units of the *campestris-multiflora* complex of the genus *Luzula* have been carried out, and the material has been investigated in the first as well as the second hybrid generations. The taxonomical units used for the investigation originate from the following geographical areas: East Asia, Europe, Eastern North America, Western North America, and Arctic North America. The species of the complex are referred to different levels of polyploidy, from the diploid to the octoploid level (Table 2). The true diploids have $2n=12$.

(2) The taxonomical units of the diploid level of polyploidy are all easily hybridized (Tables 3—8). The fertility of the first and second hybrid generations is fairly good (Tables 18—19), and the pairing of chromosomes at meiosis is usually only slightly reduced, but in some strain combinations meiosis is degenerated before first metaphase.

(3) All the taxonomical units of the tetraploid level of polyploidy cross easily except *L. comosa* of Western North America, which species shows reduced compatibility or incompatibility when tested with other tetraploids (Tables 11—13). Fertility of first as well as second hybrid generation is strongly reduced for all hybrids of the tetraploid level (Tables 20—21). The pairing of chromosomes at first metaphase is usually low, and in many combinations the pollen mother cells degenerate at an early stage.

(4) Hexaploid *L. multiflora* of Europe and Eastern North America cross easily, but the hybrids are generally almost sterile (Table 14). Meiosis is usually degenerated, but in one strain combination studied 16—18 bivalents have been found. Hybrids between the European strains of hexaploid *L. multiflora* are fertile with a regular meiosis. The same is true of the hybrids between Scandinavian *L. multiflora* and its alpine variety *L. frigida sensu* SAMUELSSON.

(5) Hybridization between species belonging to different levels of polyploidy is possible only between the tetraploid and the hexaploid or between the hexaploid and the octoploid level (Table 15).

(6) Hybridization between the endonuclear polyploids is possible when they belong to the same level of polyploidy. (Endonuclear polyploids of the hexaploid or octoploid levels of polyploidy have not been found in nature.) The hybrids may be fertile when the difference in chromosome structure is not too large. At meiosis the small chromosomes from the one of the parents with the higher chromosome number seem to pair

with corresponding large chromosomes originating from the parental species with the lower number of chromosomes; thus, they usually form different kinds of multi-associations (Figs. 3 *e*, 4 *e*, and *f*, 5 *e*, *g*, *h*, and *i*).

(7) From the results of the investigation certain general features applicable to all levels of polyploidy have been found: (a) Taxonomical units belonging to the same geographical area but ecologically separated are usually genetically closely related and show, as a rule, stronger affinity to each other than taxonomical units which are geographically separated. These characteristics hold also true for the endonuclear polyploids. (b) Certain taxonomical units are found in the complex which, more easily than the rest of the species, cross with other taxonomical units of their own level of polyploidy (Tables 18—21).

(8) In the hybridization experiments it has been found that species belonging to the diploid level of polyploidy are more easily crossed and the hybrids more fertile than is the case with hybrids between the tetraploid taxonomical units. An exception is the artificially-made tetraploid, *L. pallescens*, which gives comparatively fertile hybrids when crossed with the natural tetraploids.

(9) The polyploids of the complex seem to be more variable than the diploids, a phenomenon which may explain the fact that the diploid taxonomical units have usually been distinguished taxonomically as species or varieties, whereas the polyploids have generally been referred to the collective species *L. multiflora*. In spite of this fact it has been found that the tetraploid taxonomical units of different geographical areas display a higher degree of sterility in both the first and second hybrid generations than is the case with the diploids originating from corresponding areas.

(10) The taxonomical units of a geographical area are genetically closely related and morphologically characterized according to the ecology of their habitat. Thus, corresponding morphological characters of different taxonomical units reveal similar ecology of habitat but not genetical affinity.

(11) The genetical differentiation between the taxonomical units tested gradually increases from species giving full fertility in both the first and second hybrid generations (*L. pallescens* of Europe crossed with *L. bulbosa* of Eastern North America) to species with rather distinct differentiation (*L. comosa* of Western North America in combination with different races of *L. multiflora*).

(12) From the data obtained in the investigation an attempt has been

made to formulate a probable path of evolution among the investigated taxonomical units of the *campestris-multiflora* complex of the genus *Luzula* (Figs. 10 and 11).

Literature cited

BACKHOUSE, B. A. 1916. Note on the inheritance of »cossability». — J. Genetics, Vol. 6, pp. 91—94.

BEAUVERD, G. 1918. Excursions phytogéographiques aux environs de Viège et de Zermatt (Valais). — Bull. Soc. Bot. Genève, 2me Sér. Vol. 10, pp. 285—286.

BUCHENAU, F. 1898. *Luzula campestris* und verwandte Arten. — Österreich. Bot. Z. Jahrg. 48, pp. 284—285.

BÖCHER, T. W. 1950. Contributions to the flora and plant geography of West Greenland. II. — Meddelelser om Grönland, Bd. 147, Nr. 7, pp. 11—23.

EHRENDORFER, F. 1954. Phylogeny and evolutionary mechanisms in *Lepto-Galium*. — VIIIe congr. int. de Botanique, Paris, Sec. 4, pp. 82—84.

GAJEWSKI, W. 1954. Interspecific barriers in the genus *Geum* L. — VIIIe congr. int. de Botanique, Paris, Sec. 9—10, pp. 193—195.

HYLANDER, N. 1953. Nordisk kärlväxtflora. I. — Stockholm.

LINDMAN, C. A. M. 1926. Svensk fanerogamflora. — Stockholm.

NORDENSKIÖLD, H. 1945. Cyto-genetic studies in the genus *Phleum*. — Acta Agricult. Suecana, 1, pp. 1—138.

— 1949. The somatic chromosomes of some *Luzula* species. — Bot. Not. 1949, pp. 81—92.

— 1951. Cyto-taxonomical studies in the genus *Luzula*. I. — Hereditas 37, pp. 325—335.

SAMUELSSON, G. 1922. Zur Kenntnis der Schweizer Flora. — Vierteljahrsschrift d. Naturforsch. Gesellschaft in Zürich, 67, pp. 240—247.

ST. JOHN, H. 1936. New and noteworthy northwestern plants. VI. — Madroño, Vol. 3, pp. 219—221.

Contents

Introduction 7
Technique 16
Investigation of hybrids between the 12-chromosomal species 17
Hybrids between 12-chromosomal diploids and endonuclear polyploids of the diploid level 33
Hybrids between species belonging to the tetraploid level of polyploidy 37
Hybrids between two geographical races of the hexaploid *L. multiflora* 47
Hybrids between different levels of polyploidy 50
Naturally occurring hybrids between species or chromosomal races of the *campestris-multiflora* complex 51
Discussion and conclusions 53
Summary 71
Literature cited 73

THE GENETIC EFFECTS OF CHRONIC GAMMA IRRADIATION IN BARLEY

By *N. NYBOM, Å. GUSTAFSSON, I. GRANHALL,* and *L. EHRENBERG*
BALSGÅRD AND STOCKHOLM, SWEDEN

I. INTRODUCTION

UNTIL recently mutations in crop plants have been primarily induced by acute irradiations of seeds, pollen grains or dormant buds. There appear to be distinct differences as to the modes of action and the relative efficiencies of γ-rays, X-rays, neutrons, and other radiations. This fact, as well as differences in rates and types of mutations produced, has been illustrated in the volume »Mutation research in plants» (Acta Agric. Scand., Vol. IV: 3, 1954). As a means of completing and improving the methods of irradiation, there was in 1952 erected a ^{60}Co-source at the Balsgård Fruit Breeding Institute with the special purpose of inducing genetical changes during different stages of the growing plants.

Originally the activity of the source was 2 Curies. In 1953 it was increased to 20 Curies. Another ^{60}Co-source, used for genetic research and plant-breeding, was erected in 1955 at the Forest Research Institute. close to Stockholm, and has a strength of 80 Curies.

Although planned for the induction of bud sports in fruit-trees the gamma-source at Balsgård has become useful for the artificial production of mutations in crop plants, too, as well as for studies on the influence of continuous radiation on biochemical processes in the living organism. In this connection we want to refer to similar American studies by SPARROW and SINGLETON (1953), SINGLETON *et al.* (1955), and SPARROW *et al.* (1955) as well as to works of MIKAELSEN (1955) in Norway, and to the publications by GRANHALL, EHRENBERG, and BORENIUS (1953), EHRENBERG, GRANHALL, GUSTAFSSON, and NYBOM (1954), where some Swedish experiments are described. Biochemical studies were undertaken by JAARMA (1955), illustrating the change of sugar metabolism under the influence of chronic irradiation.

Originally the main advantage of a γ-source was considered to be the detection of special sensitive or insensitive stages during the vegetation period. In these stages mutation rates may vary considerably as com-

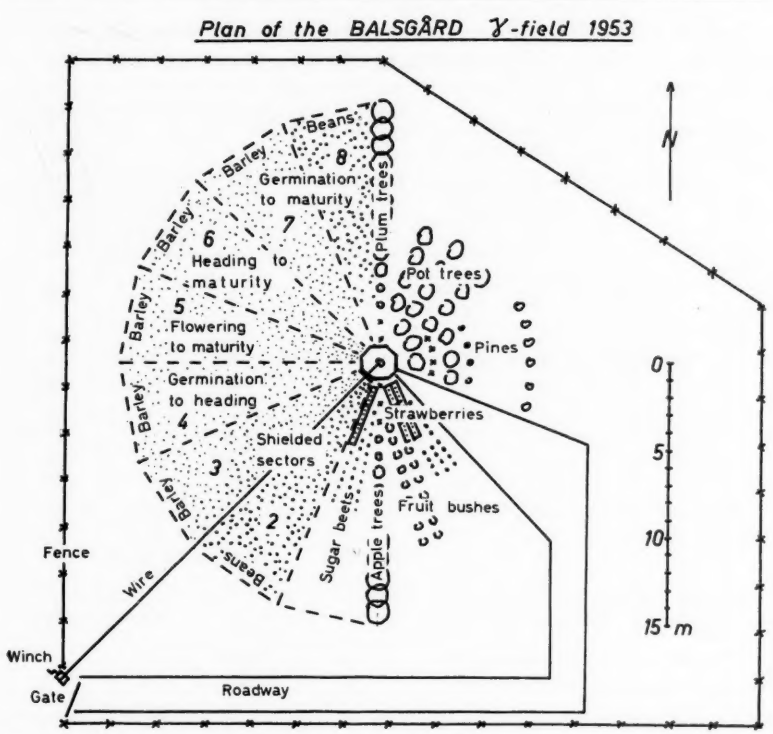


Fig. 1. Plan of the Balsgård γ-field in 1953. Most of the fruit trees and bushes indicated were such that were irradiated at the source for varying time and then moved to permanent growth places. There is an additional fence outside the one shown.

pared to the effects in seeds, buds or pollen grains, or one might expect special types of mutation or changes at specific loci to be induced predominantly during certain stages. Barley being well fitted for such studies was included among the species studied. Irradiated material has now been followed for the three generations necessary for the genetical analysis.

II. METHODS OF RESEARCH

Seeds of barley (the two-row variety *Bonus*) were sown in concentric rows around the 20-Curie ^{60}Co-source in the spring of 1953. The irradiated circular field was split up in sectors (Fig. 1), which were either irradiated continuously (sector 7), or were protected by means

TABLE 1. *Genetical changes in plants irradiated during the entire vegetation period.*

γ_1; Balsgård 1953				γ_2; Svalöf 1954	γ_3; Green-house data 1954—55		
1	2	3	4	5	6	7	8
Plot No.	Distance from the source, m	Daily γ-dose r-units	Average plant fertility	γ_2-spikes with < 90 % fertility	γ_2-spikes giving normal offspring	γ_2-spikes with chlorophyll mutations	‰ γ_2-spikes with chlorophyll mutations
1—2	1,5—2,3	270—110	all plants dead	—	—	—	—
3	2,6	90	0	—	—	—	—
4	3,0	67	21	71	340	19	53
5	3,4	51	45	45	792	51	60
6	3,9	40	55	38	1232	71	55
7	4,3	32	64	41	1126	13	11
8	4,8	27	59	49	830	30	35
9	5,2	22	64	19	858	45	50
10	5,6	19	64	17	1355	23	17
11	6,2	16	69	19	1261	22	17
12	7,7	10	74	16	1282	2	2
13	9,7	6	68	5,5	1186	12	9
14	11,7	4	67	9,6	1107	2	2
15	13,8	3	71	9,3	1245	1	1
Shielded sector, Balsgård: ca. 75				2,8	2409	11	5
Controls grown at Svalöf: ca. 95				0,1	8966	6	0,7

of movable lead and iron-concrete shields during certain parts of the vegetation period (sectors 3—6). The irradiation was only interrupted for very short intervals of watering and inspection.

Two kinds of control material were available. The first consisted of plants grown in sector 3 of the gamma field, shielded from irradiation. As may be seen in Table 1, the plants of this sector nevertheless received a good deal of radiation. The shielding being calculated to absorb 99,9 % of the primary beam, the radiation must have been chiefly due to radiation scattered from surrounding materials. The principal control material consisted of plants grown at Svalöf, 100 kilometres from the gamma source. Though of course not wholly identically treated, this material is in genetic respects fully comparable to the Balsgård material.

The dose-rate (in air) was determined at a distance of 1 metre with a Victoreen-type air-walled ionization chamber to 25 r per hour, and the dose-rates at other distances were calculated according to the square

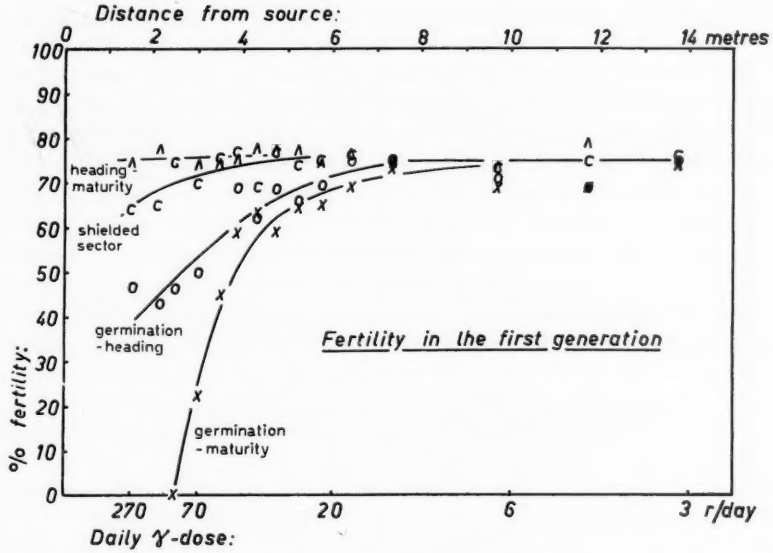


Fig. 2. Diagram showing the seed sterility brought about in the first generation in plants standing at various distances from the source and irradiated during different parts of the vegetation period.

law. At its maximum the screening effect of air and shadowing plants was about 5 per cent, and no correction was thought necessary. Since the dimensions of a developing spike is variable and not large compared to the range in tissue of ionizing electrons — in average about 2 mm — the tissue dose (in rep) might deviate somewhat from the air dose.

After ripening the plants were harvested in plots from the centre, near the source, outwards towards the periphery, circa 15 metres away. The harvested plants correspond in part to the X_1-generation after treatment of seeds with acute irradiation and will here be called the γ_1-generation. The fertility of these first-generation plants was determined in the fall of 1953 (*cf.* Fig. 2). The threshed seeds, registered as to plant and spike descendance, were sown in the experimental fields of Svalöf in 1954. Most of the γ_2-spikes then obtained were sown in the green-house in the winter of 1954—55 according to standard methods (NYBOM, 1954) for analysis of chlorophyll mutations in the γ_2-generation. One spike per γ_2-plant was sown in the field in 1955 for analysis of viable mutations.

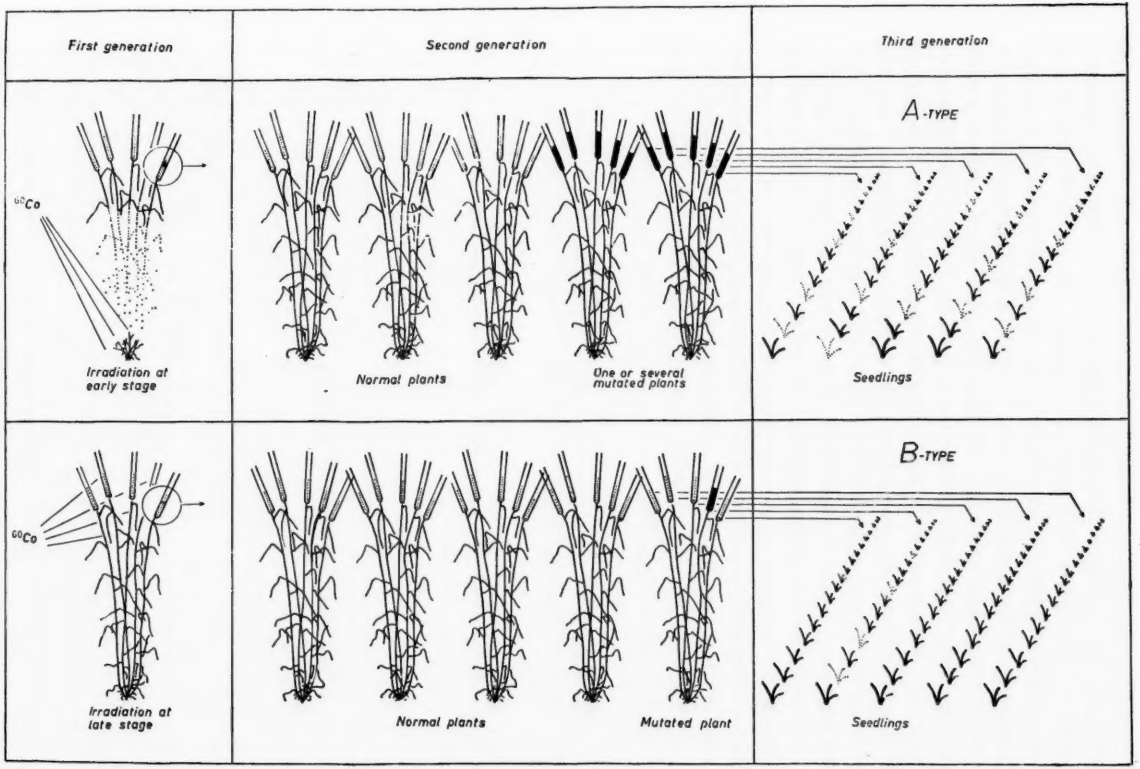


Fig. 3. Schematical presentation of the different mode of appearance of the mutants in the later generations, according to whether they are induced at an early or at a late stage in the first generation. In the second generation 5 plants are shown, coming from one γ_1-spike. They may vary in number, according to the degree of sterility in the γ_1-plants.

It is necessary to harvest the γ_2-plants individually, not only the γ_2-spikes, for the following reasons. Under continuous irradiation there are two chief periods of treatment, one lasting from the sowing of the seeds up to the time of fertilization, the other lasting from fertilization to the time of harvest. If an induced mutation has occurred sufficiently early in development, the resulting γ_1-seed will become genetically uniform, heterozygous for one and the same mutation (called A-type in Fig. 3). The γ_2-plants resulting from such seeds will then be genetically uniform (not chimaerical) throughout, either heterozygous or homozygous. If, on the other hand, the mutation occurs late in ontogenesis, after fertilization has already taken place and the embryos of the spike have become multicellular, just one seed of the spike (and of the plant) will be heterozygous for the induced mutation and it will then be chimaerical for the mutation in question (the B-type; *cf.* Fig. 3). This state of things will lead to contrasting results in the mutation analysis. In the case of the A-type whole γ_2-plants, but with regard to the B-type single γ_2-spikes or even parts of spikes will be mutated.

III. EXPERIMENTAL RESULTS

1. The whole vegetation period irradiated

The normal control material of barley had, as is generally the case, an average fertility of 95 %, *i. e.*, one kernel in twenty is aborted. The shielded control material at Balsgård had an average spike fertility of circa 75 % (Fig. 2), a difference in part due to environmental factors, however. After a daily dose of 110—270 r-units per day all plants of sector 7 were killed. At the highest doses that permitted survival of the plants the radiation resulted in a complete sterility, which gradually decreased to the 75 % fertility of the control. The »threshold for reduced fertility in the first generation» has for several years been found to lie between 6 and 8 metres, *i. e.*, at a daily dose of 10—20 r-units.

It is interesting to note the difference in maximum sterility after chronic irradiation of growing plants and acute irradiation of dormant seeds. In the latter case the fertility of the X_1-spikes never decreases below 40—50 %, owing to the »intrasomatic elimination» in the damaged plants (*cf.* EHRENBERG *et al.*, 1953). Dividing the irradiation into part-periods (*cf.* Fig. 2) shows that it is the irradiation during the early embryo formation that is responsible for the complete sterility.

In the second generation (γ_2) of the treated material (sector 7) there are many plants with less than 90 % fertility. This partial sterility is due to inherited chromosome changes, chiefly translocations in the heterozygous condition, which cause a spike sterility of 10—30 %, when one of them is present (*cf.* NYBOM, 1950; HAGBERG, 1954). The proportion of plants showing this degree of sterility steadily increases with the dosage; from less than 3 per cent in the shielded sector up to more than 70 per cent after 70 r-units per day.

It is important to note that the irradiated plots show a distinct increase in the frequency of sterile spikes even after daily gamma-doses of just a few r-units.

With regard to the chlorophyll mutations, used as test mutations in this study (Table 1), just a few instances were found in the γ_2-generation, indicating that most of the mutations were induced at a stage later than the separation of the male and the female organs of the γ_1-flowers.

The control material grown at Svalöf showed 6 cases of spontaneous chlorophyll mutations among slightly less than 9.000 spike offsprings, *i. e.*, less than one per mille. Thus in the shielded sector at Balsgård the mutation frequency had risen several times, which could not be attributed to spontaneous mutation.

Whether the mutation rate, within the whole range of doses, is a linear function of dose is difficult to conclude from the present material. The fundamental fact is evident, however, that even after the smallest doses used there is a substantial increase in mutation frequency. Already after about 15 r/day the rate is increased some 10—15 times. This is in contrast to the results presented by SINGLETON (1954) for endosperm characters in maize. According to him, there is »a threshold of dosage required to raise the mutation rate from the spontaneous level to a detectable increase over that level». He found the threshold to be quite high, between 10 and 40 r per day in different experiments.

It is difficult to judge the significance of these deviating results. It seems, however, that the endosperm deficiencies studied in maize show several peculiarities, necessary to take into consideration, among other things a spontaneous mutation rate at least 100 times higher than what is usually regarded as »normal» and a mutation coefficient, above the threshold, which is likewise far above that found in most other materials. The chlorophyll mutations studied by us behave in these respects more like »normal factor mutations» (*cf.* NYBOM, 1955).

In this connection references may be made to the concordant results of UPHOFF and STERN (1949) who did not find any threshold in *Drosophila* after low dose rates. A similar result was published by SAX (1950) using chronic irradiation of *Tradescantia* pollen.

2. Different stages irradiated

The additivity of the radiation injury is obvious also from the data of Table 2, where the mutation frequencies of four different sectors of the gamma-field have been compared. Only three doses, 40—67 r/day

TABLE 2. *The effect of irradiation during various parts of the vegetation period.*

Sector	Irradiation		γ_2-spikes analysed	Number of mutations	Mutations per γ_2-spike and r-unit	% mutations representing		
	Period	Days				A-type	B-type	Uncertain [1]
4	Germination to heading	41	2366	64	10×10^{-6}	56	33	11
5	Flowering to maturity	27	3067	41	9×10^{-6}	2	94	4
6	Heading to maturity	43	2944	98	12×10^{-6}	7	85	8
7	Germination to maturity	85	2505	141	12×10^{-6}	18	69	13

[1] Classification impossible since too few spikes per plant were formed.

have been used for computing the mutation coefficients. The average mutation rate per γ_2-spike progeny and r-unit is 12×10^{-6}, when the plants were irradiated for the entire vegetation period (sector 7). In the first half of the period (sector 4), from germination to heading, the rate was of the same order, 10×10^{-6}. During the second part of the vegetation period (sector 6), from heading to maturity, again a value of 12×10^{-6} is found. In sector 5, finally, the irradiation lasted for a shorter time, from flowering to maturity. Here the mutation rate was slightly lower, *viz.* 9×10^{-6} per r-unit and spike, but the difference is not significant. These results indicate a complete proportionality between dosage and mutation rate, independent of the stage of treatment, at least when the life cycle is divided up into fairly long periods like here.

As mentioned in the technical description, there are two types of mutated plants, the A-type and the B-type (*cf.* Fig. 3). The A-type mutations are induced already before fertilization. Consequently the surviving γ_2-plants will be uniformly heterozygous — or normal — throughout and all spikes of the heterozygous ones will segregate for the mutation. The B-type mutations, which are induced after fertilization, will give rise to γ_1-seeds which are genetically chimaerical. Thus some spikes of a γ_2-plant will remain normal while others are heterozygous for the mutation.

When the irradiation is carried out during different parts of the vegetation period differences in the distribution of A- and B-types are obtained in accordance with the interpretation of their mode of origin. If the irradiation occurs during the first half of the vegetation period (Table 2, sector 4) 56 per cent of all mutations represent the A-type. When the irradiation takes place in the latter half of the period (sector 6), however, no more than 7 per cent belong to this type. When the irradiation is concentrated to the last 27 days the proportion of A-types has decreased still further, down to 2 %. The irradiation over the entire period gives an intermediate value, 18 % (sector 7).

If the mutations actually recorded are divided up into these two types it is found that the A-type mutations give a segregation ratio in γ_2 close to 3 : 1, producing on an average 23,6 % recessive seedlings. Plants belonging to the B-type, on the other hand, give a distinct deficiency of recessives, *viz.* only 15,0 % such seedlings.

Probably the disturbed segregation ratio of the B-type is conditioned by accompanying chromosomal abnormalities, which — in the case of the A-type mutations — were filtered away before or at meiosis in γ_1.

There is a slight indication that the phenotypical type of mutation, whether *albina* or *viridis* (white or light green; *cf.* GUSTAFSSON, 1940; NYBOM, 1955), correlates with its mode of origin, so that relatively more *albinas* arise when the plants are irradiated early in the vegetation period, whereas *viridis* predominates when the post fertilization period is irradiated. The data are not significant, however, but conform with the ideas presented by EHRENBERG (1955, p. 142).

The mutation rates in the case of chronic irradiation appear to be close to the rates obtained when seeds are given acute γ-rays (*cf.* EHRENBERG and SAELAND, 1954) or X-rays (EHRENBERG *et al.*, 1953). However, the rates of acute irradiations then refer to the direct offspring of the X_1-spikes, whereas the chronic irradiations refer to the offspring of the γ_2-spikes to which the X_1- spikes are nearest, but not

completely, corresponding. To be made more comparable the material should in both cases be followed still a generation further to unveil also the cases that have remained heterozygous in X_2 and γ_2, respectively. This would probably lead to a somewhat larger increase in the mutation coefficients corresponding to the acute irradiation.

MIKAELSEN has in his report of 1955 arrived at the view that chronic irradiations would give mutation rates considerably higher than after acute irradiation. In our material this is not so.

IV. CONCLUSIONS

From this series of studies it is possible to conclude:

(1) that with the lowest dose-rates used, 5—10 r-units per day, the genetic effects of chronic irradiation are truly additive, both with regard to chromosomal re-arrangements leading to sterility in the second generation as well as to recessive mutations appearing in the third generation;

(2) that consequently, when barley is exposed to chronic irradiation, there seems to be no threshold below which the irradiation becomes ineffective;

(3) that acute and chronic irradiations, as far as they are comparable, produce similar mutation rates;

(4) that no special sensitive period could be detected in barley, at least not with our way of dividing the vegetation period into a few major units; and

(5) that when growing plants are irradiated the mode of appearance in the offspring and perhaps also the type of the mutations varies with the stage of origin during the life cycle.

Whether chronic irradiation has any distinct advantage in plant-breeding over the traditional methods with acute treatments cannot be decided from the present results. But considering the differences in the »mutation spectrum» brought about by the various radiations we regard it advisable to try γ-rays along with the other ones.

Literature cited

EHRENBERG, L. 1955. Factors influencing radiation induced lethality, sterility, and mutations in barley. — Hereditas 41: 123—146.

EHRENBERG, L., GRANHALL, I., GUSTAFSSON, Å., and NYBOM, N. 1954. Acute and chronic ^{60}Co gamma irradiation of plants. — Radioisotope Conf., 1954, 1:391—396.

EHRENBERG, L., GUSTAFSSON, Å., LUNDQVIST, U., and NYBOM, N. 1953. Irradiation effects, seed soaking and oxygen pressure in barley. — Hereditas 39: 493—504.

EHRENBERG, L. and SAELAND, E. 1954. Effects of pile radiation on barley seeds. — J. Nuclear Energy 1: 150—169.

GRANHALL, I., EHRENBERG, L., and BORENIUS, S. 1953. Experiments with chronic gamma irradiation on growing plants. — Bot. Notiser 1953: 155—162.

GUSTAFSSON, Å. 1940. The mutation system of the chlorophyll apparatus. — Lunds Univ. Årsskr., N. F., Avd. 2, 36: 1—40.

GUSTAFSSON, Å. *et al.* 1954. Mutation research in plants. — Acta Agr. Scand. 4: 359—639.

HAGBERG, A. 1954. Cytogenetic analysis of erectoides mutations in barley. — Acta Agr. Scand. 4: 472—490.

JAARMA, M. 1955. Effects of chronic gamma irradiation on some sugars in *Vicia faba*. — Suomen Kemistilehti B 28: 40—45.

MIKAELSEN, K. 1955. Genetic effects of chronic γ-radiation from Co^{60} in plants. — Intern. Conf. Peaceful Uses Atomic Energy, Geneva, P/890.

NYBOM, N. 1950. Studies on mutations in barley. Superdominant factors for internode length. — Hereditas 36: 321—328.

— 1954. Mutation types in barley. — Acta Agr. Scand. 4: 430—456.

— 1955. The pigment characteristics of chlorophyll mutations in barley. — Hereditas 41: 483—498.

SAX, K. 1950. The cytological effects of low-intensity radiation. — Science 112: 332—333.

SINGLETON, W. R. 1954. The effect of chronic gamma radiation on endosperm mutations in maize. — Genetics 39: 587—603.

SINGLETON, W. R., KONZAK, C. F., SHAPIRO, S., and SPARROW, A. H. 1955. The contribution of radiation genetics to crop improvement. — Intern. Conf. Peaceful Uses Atomic Energy, Geneva, P/110.

SPARROW, A. H. and GUNCKEL, J. E. 1955. The effects on plants of chronic exposure to gamma radiation from radio-cobalt. — Intern. Conf. Peaceful Uses Atomic Energy, Geneva, P/266.

SPARROW, A. H. and SINGLETON, W. R. 1953. The use of radiocobalt as a source of gamma rays and some effects of chronic irradiation on growing plants. — Amer. Nat. 87: 29—48.

UPHOFF, E. and STERN, C. 1949. The genetic effects of low intensity irradiation. — Science 109: 609—610.

THE ROLE OF THE KINETOCHORE IN CHROMOSOME ORGANIZATION

By *A. LIMA-DE-FARIA*

INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION

CYTOLOGY developed as a tributary science of genetics, and this has had the undesirable consequence that as a rule chromosomes have been studied only to the extent that the questions put by the genetic analysis demanded a knowledge of their behaviour. With the exception of BĚLAŘ, BELLING, BRIDGES and HEITZ, few workers have carried out a systematic study of the fine structure of the chromosomes. The result has been a constant accumulation of fortuitous and incomplete descriptions of chromosomal details which has grown without a framework of its own, and which at times has displayed a most confused picture. The kinetochore, owing to its importance, reflects better than any other chromosome section this situation. Although hundreds of references have been made to this region it can be seen from the works of ÖSTERGREN (1951), CAMARA (1953), SCHRADER (1953) and WHITE (1954) that until quite recently, we still lacked a criterion to delimit the kinetochore at such an important stage as metaphase I of meiosis; we were still searching after its structural components; its DNA content was put in doubt; its division cycle erroneously interpreted, and its genetic constitution overlooked. This period is now approaching a turning point.

By the use of modern techniques it has been possible to obtain results that permit us to study the kinetochore with precision and in this way to extract from the mass of accumulated data a coherent picture of the structure and behaviour of this chromosome section. The kinetochore may be seen in large and sharp photomicrographs as distinctly as any other chromosome segment. Its structure, delimitation, division cycle, degree of heteropycnosis, chemical constitution, structural and functional variability and genetic behaviour can now be laid on an accurate basis. Moreover, the role of the kinetochore and of other chromosome regions in chromosome organization turns out to be more significant than was suspected before, and we are at present facing a situation

where the study of the chromosome structure and organization is putting its own problems independently of those raised by genetic analysis.

This paper deals solely with the localized kinetochore or centromere. Dubious observations, results based on old-fashioned techniques and works not dealing directly with the subjects treated were excluded from the literature cited.

II. STRUCTURE AND PROPERTIES OF THE KINETOCHORE

1. The fundamental pattern

With the exception of the polytene chromosomes of certain tissues of the Diptera, pachytene is in plants and animals the stage where the chromosomes display their most detailed structure, and diplotene is, in the oocyte nuclei of such vertebrates as Amphibia, fishes, reptiles and birds, a stage where the chromosomes also exhibit a most differentiated structure. At both stages the chromosomes become especially large. In conformity with these two features it is to be expected that the kinetochore reveals its most detailed structure at these stages.

The kinetochore of the pachytene chromosomes of rye (LIMA-DE-FARIA, 1949 a) was found to consist of the following components (considering the two paired chromosomes of each pachytene bivalent and starting from one end of the kinetochore): a pair of almost unstained fibrillae, a chromomere pair, a pair of deeply stained fibrillae, another chromomere pair and again an almost unstained fibrilla pair (see Figs. 9, 46 and 48). These components constitute three natural zones: the exterior zone composed by the two almost unstained fibrilla pairs, the median zone composed by the two chromomere pairs, and the interior zone where the deeply stained fibrilla pair is seen. A subsequent study of the rye kinetochore at the same stage (LIMA-DE-FARIA, 1955 c) showed that one (Fig. 47) or in some cases two chromomeres may be observed at the interior zone. The presence of these chromomeres demonstrates that the interior zone has also a compound structure, consisting of minor chromomeres that become visible under favourable conditions.

In the lampbrush chromosomes of oocyte nuclei of *Triturus viridescens* (GALL, 1954), the kinetochore can be seen to have a similar pattern. Here in the newt, at diplotene, it has the very large size of 10×2 micra and it consists of the following components (starting from

one end of the kinetochore): a fibrilla, a medium size chromomere, a fibrilla, another medium size chromomere, a fibrilla, a very long and broad chromomere, again a fibrilla, a medium size chromomere, a fibrilla, a medium size chromomere, and a fibrilla.

The very large central chromomere is differentiated into two regions, a peripheric and a central one. Under favourable conditions up to six minor spherical bodies can be seen surrounding its central region. This region is also the principal part of the large chromomere that shows attachment when two kinetochores happen to come into contact. The chromomeres are connected by segments of a fine chromonema — the fibrillae — which are almost transparent. The observations were made in unfixed material under phase-contrast (GALL, 1954).

In the newt, as in rye, the kinetochore consists of three natural zones: the exterior zone composed of the six almost transparent fibrillae and the four medium size chromomeres, the median zone composed of the two external regions of the large chromomere, and the interior zone where the numerous smaller bodies are found.

In rye, the exterior zone was not seen to be composed of minor elements but it was expected that it would show a compound structure since both its parts measure not less than one micron. In *Triturus* both parts amount to about five micra and within this space, six fibrillae and four chromomeres can be seen. Although this is a very differentiated pattern, this zone has, as in rye, the least conspicuous appearance of the three.

We can at present describe the fundamental pattern of the kinetochore as follows.

(1) Exterior zone. The least conspicuously differentiated. Composed of almost unstained fibrillae (rye) or of almost transparent fibrillae and medium size chromomeres (newt). Length about one and five micra respectively.

(2) Median zone. The most conspicuously differentiated. Composed of two distinct chromomere pairs (rye) or of the external regions of the very large chromomere (newt). Length about one and two micra respectively.

(3) Interior zone. Composed of small chromomere pairs (rye) or of the central region of the very large chromomere (newt). Length about half a micron and two and a half micra respectively.

Every zone of the kinetochore has a length that is equivalent to the diameter of the short arms of the mitotic metaphase chromosomes of many plant and animal species and each zone has a compound structure.

2. Generality of occurrence of the fundamental pattern

The structure described for rye has been found at pachytene to be essentially alike in every chromosome of the normal complement (LIMA-DE-FARIA, 1952 a) and in the B chromosome types studied in this species (LIMA-DE-FARIA, 1949 a, 1952 a, MÜNTZING and LIMA-DE-FARIA, 1952, 1953, and LIMA-DE-FARIA, 1955 c). A similar structure can be seen in the pachytene chromosomes of *Agapanthus umbellatus* (LIMA-DE-FARIA, 1950, 1955 c) and was also fortuitously reported by DARLINGTON (1935) in a pachytene chromosome of *Fritillaria Eggeri*. The presence at the kinetochore of one or two chromomeres or chromomere pairs joined to each other and to the chromomeres of the arms by fibrillae has been reported at pachytene in: *Antirrhinum majus* (ERNST, 1939), *Fritillaria imperialis*, *F. verticillata*, *F. pontica*, *F. oranensis* (DARLINGTON, 1935), *Tulipa Orphanidea* (UPCOTT, 1937), *Veltheimia viridifolia* (COLEMAN, 1940), *Agapanthus umbellatus* (BELLING, 1928 b, DARLINGTON, 1933, GEITLER, 1933), and *Kniphofia aloides* (DARLINGTON, 1933).

At diplotene, in lampbrush chromosomes, GALL (1954) observed in *Amblystoma tigrinum* (a salamander) a kinetochore structure essentially like that described for *Triturus*.

The same type of kinetochore pattern is found at prophase and metaphase of mitosis. TJIO and LEVAN (1950 a and b) after pre-treatment of root tips with oxyquinoline found the kinetochore of metaphase chromosomes of *Scilla sibirica* to consist of an almost unstained fibrilla pair (exterior zone), a chromomere pair (median zone), an almost unstained fibrilla pair (interior zone) and again a chromomere pair, and an almost unstained fibrilla pair, each chromomere and fibrilla corresponding to a sister chromatid. In these chromosomes the exterior and interior zones were not found to be microscopically compound, but the same fundamental pattern is seen. TJIO and LEVAN (1950 b) ascertained the general occurrence of this structure at prophase and metaphase of mitosis by finding it after the same treatment in: *Tradescantia bracteata*, *Scilla Strangwaysii*, *S. undulata*, *Hyacinthus orientalis*, *Allium cernuum*, *A. Cepa*, *A. sphaerocephalum*, *Veltheimia viridifolia*, *Sauromatum guttatum*, *Amorphophallus rivieri*, *A. Zeylanica*, *Haemanthus albiflorus*, *Vallota purpurea*, *Amaryllis* sp., *Crinum amabile*, *Cyrtanthus lutescens*, *Nerine humilis*, *Hymenocallis lacera*, *Hordeum vulgare*, *Secale cereale*, *Triticum monococcum*, and *Vicia faba*. These plants belong to such different families as the Commelinaceae, Liliaceae, Araceae, Amaryllidaceae, Gramineae, and Papilionaceae. This study was ex-

tended after the same oxyquinoline treatment, to *Phleum echinatum* (ELLERSTRÖM and TJIO, 1950), *Nothoscordum fragrans* (KURITA, 1953) and, after quinoline treatment, to the mouse (TJIO and LEVAN, 1954) with similar results.

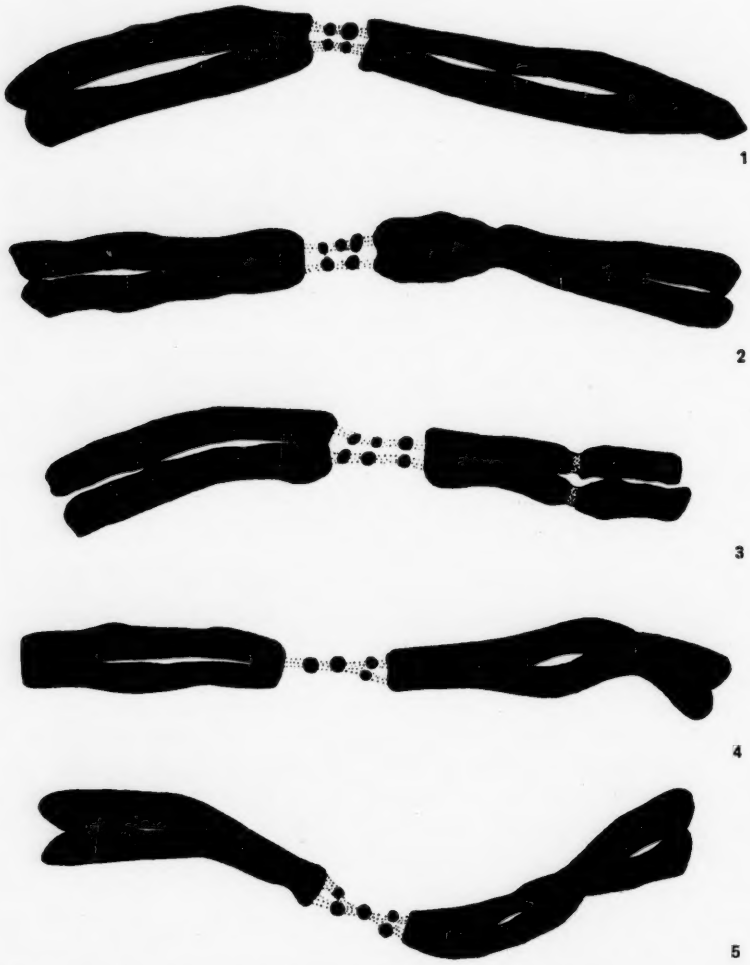
In untreated metaphase or early anaphase of mitosis a chromomere united to the arms by weakly stained fibrillae was depicted in: *Trillium grandiflorum* (SHARP, 1934, 1943), *Fritillaria Raddeana* (KOSLOV, 1937), *Galtonia candicans* (NAWASCHIN, 1927, at pollen mitosis) and *Tradescantia gigantea* (PROPACH, 1940, also at pollen mitosis). Also in untreated mitotic chromosomes of *Ulophysema öresundense*, a crustacean, (MELANDER, 1950) the kinetochore of each sister chromatid was seen to consist of a fibrilla pair, and two closely attached chromomere pairs, the chromosomes being telocentric as in the mouse.

I have now found that the interior zone also displays a compound structure at mitosis. In root tips of *Hyacinthus orientalis*, treated with 0,2 % colchicine for 8 hours, the chromosomes show at early metaphase the two chromomere pairs of the median zone and in addition one chromomere or chromomere pair located at the centre of the kinetochore (Figs. 1—6 and 8).

All the figures and photomicrographs of this paper were made from permanent preparations mounted in Canada balsam. The chromosomes of Figs. 1—8, 23—25, 34, 35, 37, 38 were stained with aceto-orcein after fixation in acetic-alcohol 1 : 3 for 10—30 minutes, those of Figs. 9, 15—17, 39, 41—46 were fixed and stained according to the aceto-carmine technique described in previous papers, those of Figs. 10—14, 18—22, 26—33, 40 were stained by the Feulgen test (see below) and that of Fig. 36 with fuchsin-sulphurous acid after fixation in acetic-alcohol 1 : 4 for 10 minutes. No spindle structure is visible in any of the preparations.

The plants used in this investigation were obtained from the sources referred to below: *Hyacinthus orientalis* and *Fritillaria meleagris* (Normanns Seed Co. Lund), *Tradescantia virginiana* (Botanical Gardens of Lund and Copenhagen), *T. bracteata* (Institute of Genetics, Copenhagen), *Galtonia candicans* (Botanical Garden, Stockholm), *Allium Cepa* and *Vicia faba* (commercial varieties), *Agapanthus umbellatus* and *Rhoeo discolor* (Botanical Garden, Lund). The chromosomes of *Secale cereale* are from the material used in my study (LIMA-DE-FARIA, 1952 a) and the males of *Mecostethus grossus* were collected at Stångby (near Lund) with the collaboration of Dr. A. LUNDQVIST and Mr. G. ISING to whom I am indebted. I am also indebted to Agronomist R. HOCHBERGS and Mrs. I. M. HÄGG for technical assistance.

Due to the particular behaviour of the proximal regions of the arms (Figs. 39 and 40) there is at present a criterion by which the kinetochore can be delimited at metaphase and anaphase I of meiosis (LIMA-DE-FARIA, 1955 d). Such delimitation was a prerequisite for the recognition of the same fundamental pattern of the kinetochore at these stages.



Figs. 1—5. *Hyacinthus orientalis*. Colchicine treated root tip chromosomes at early metaphase showing chromomere structure at kinetochore. — Fig. 1. A chromosome with the common type of structure observed at the kinetochore consisting of three fibrillae and two chromomeres per chromatid. — Figs. 2—5. The compound nature of the interior zone is revealed by the presence of one chromomere or one chromomere pair at the centre of the kinetochore. — All camera lucida drawings. — ×5900.

This was then found in *Tradescantia virginiana* (LIMA-DE-FARIA, 1955 d).

The folding of the kinetochore at these meiotic stages was considered earlier by SCHRADER (1939) but no attempt was made to demonstrate it. This remained for a long time a serious obstacle to a precise analysis of the kinetochore. There are two main difficulties in the study of the folding of the kinetochore at metaphase and anaphase I. One is due to the close juxtaposition of both arms at their proximal regions, and the other to the fact that once such a folding is observed in a chromosome, it cannot be easily distinguished from a kinetochore in which the exterior zone is divided. It was expected that in cases where free chromatids would be available at anaphase I these difficulties would be circumvented. In *Secale cereale* several cases of free sister chromatids were found. These free sister chromatids resulted from bivalents showing difficulty of separation with the consequent precocious detachment of one of the chromatids of a chromosome and from univalents that divided during the first division. In these free sister chromatids the arms are not in so intimate a contact and the kinetochore can be clearly seen to be folded, making the connection between the short and long arm (LIMA-DE-FARIA, 1955 d). I have now found that in tetravalents of *Tradescantia virginiana*, where the arms of each chromosome — due to the zigzag arrangement of the chromosomes — may become slightly separated from each other, the folding of the kinetochore is clearly displayed at metaphase I (Figs. 19—22). In bivalents the folding is more difficult to observe due to the parallel orientation of the arms, but in a few cases, where the arms happen to lie slightly separated, it may then be seen with equal distinctness (Figs. 10 and 11, upper kinetochore). In such chromosomes (as may be seen especially in Figs. 10 and 19) the short arm is attached to one half of the kinetochore in upward direction, consisting of a weakly stained fibrilla (exterior zone), one chromomere or spherule (median zone), a weakly stained fibrilla (interior zone); the other half of the kinetochore in downward direction, is formed by the same three zones, and is attached to the long arm. At mitosis where the arms of each chromosome are usually widely separated the folding can be seen without difficulty at late metaphase (or early anaphase) in organisms such as *Galtonia candicans* (Figs. 23—25).

That the interior zone of the kinetochore has also a compound structure at metaphase I of meiosis can now be shown. In Figs. 26—31 a chromosome belonging to a bivalent or multivalent of *Tradescantia virginiana* is represented. Besides the chromomeres of the median zone one

or two chromomeres (Fig. 21) may be seen at the interior zone and in some cases a third fibrilla surmounts the chromomeres of the interior zone (Figs. 21, 26, 27, 29, 31), which shows that they are located a certain distance apart.

The structure described in *Tradescantia virginiana* chromosomes can be seen to be of general occurrence. In *Tradescantia bracteata* (Figs. 12, 13, 18, 32) and in *Mecostethus grossus*, a grasshopper (Figs. 15 and 17), the same three zones can be seen very distinctly at metaphase I. The presence at the kinetochore of a chromomere or chromomere pair united to the chromosome arms by a weakly stained fibrilla or fibrilla pair has been depicted at metaphase I or anaphase I of meiosis among other plants in: *Tradescantia reflexa* (NEBEL, 1935, SCHRADER, 1939), *T. bracteata* (MATHER, 1934, DARLINGTON, 1938), *T. virginiana* (ÖSTERGREN, 1951), *Fritillaria latifolia major* (DARLINGTON, 1940 b), *Galtonia candicans* (LA COUR, in DARLINGTON, 1937), *Trillium Smallii* (IWATA, 1940), *T. kamtschaticum* (MATSUURA, 1941), *Eremurus spectabilis* (UPCOTT, 1936), *Secale cereale* (CAMARA, 1939), *Hordeum vulgare* (CALDECOTT, 1953), *Triticum vulgare* (SMITH, HUSKINS and SANDER, 1950), *Datura stramonium*, a Solanaceae (SATINA and BLAKESLEE, 1935), *Podophyllum versipelle*, a Berberidaceae (DARLINGTON 1936), *Ranunculus nodiflorus*, a Ranunculaceae (NEVES, 1945) and *Alstroemeria* sp., an Alstroemeriaceae (LA COUR, in DARLINGTON, 1937). The structure depicted by these authors can now be seen to be essentially the same as described in detail for *T. virginiana*, but among other things, the interior zone was not represented. As could be seen in the species *T. virginiana* and *T. bracteata*, when a more detailed study of the kinetochore was undertaken this zone turned out to be present. The same happened at pachytene and at metaphase of mitosis, where the description of one or two chromomeres has been substituted by the observation of a well differentiated structure. The reason may be sought in the fact that most authors were looking for other chromosome features when they drew the kinetochore, and also in the recent employment of more adequate techniques in its investigation.

No essential difference can now be found between the structural organization of the kinetochore at metaphase and anaphase of mitosis and at metaphase and anaphase I of meiosis, a point of particular significance in connection with chromosome movements. The differences between these two groups of stages in the arrangement of the kinetochore components relative to the arms and to the spindle poles have been previously described (LIMA-DE-FARIA, 1955 d).

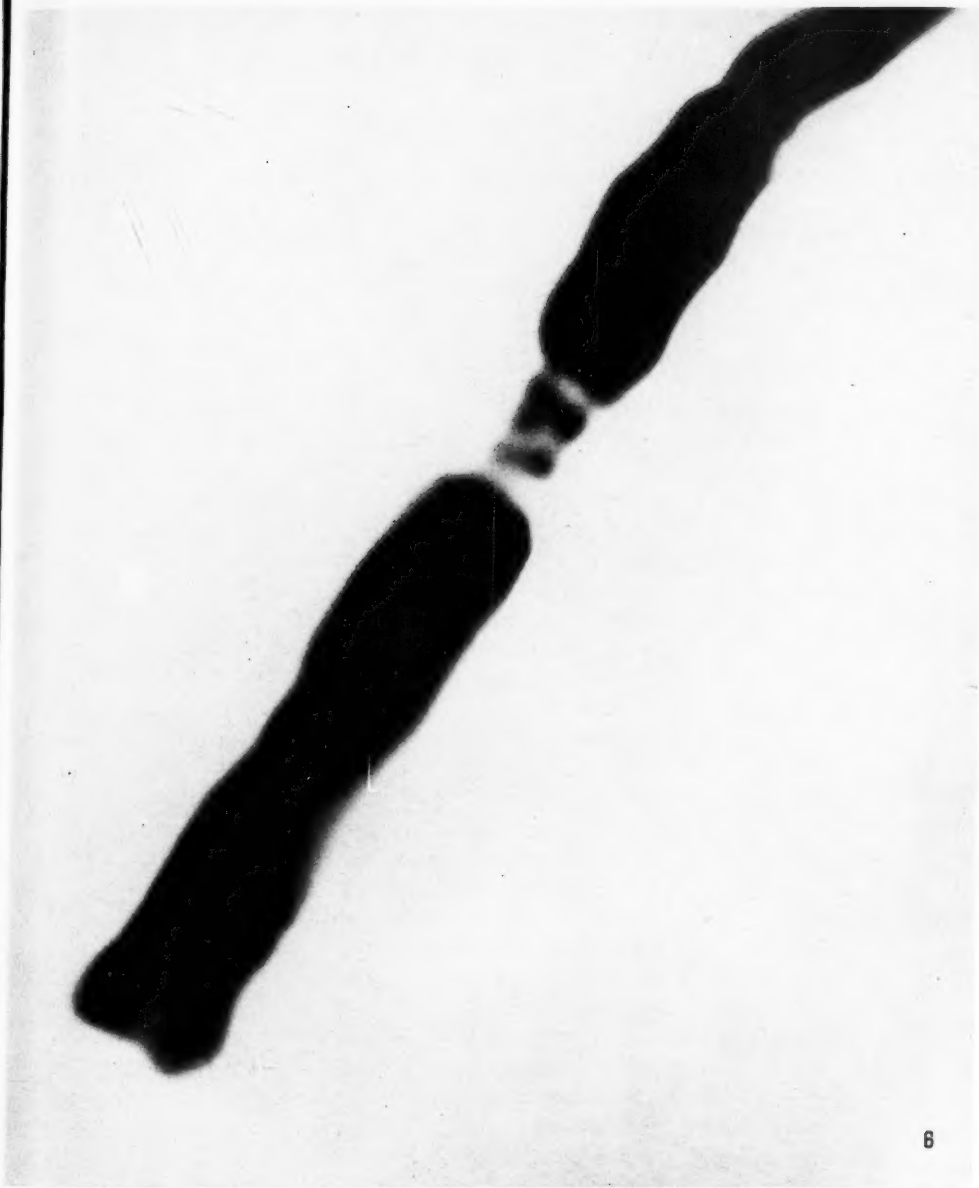


Fig. 6. ***Hyacinthus orientalis.*** **Photomicrograph of the chromosome drawn in Fig. 2. Within the square built by the four chromomeres of the median zone, one chromomere may be seen at the interior zone of the left chromatid. — ×10700.**

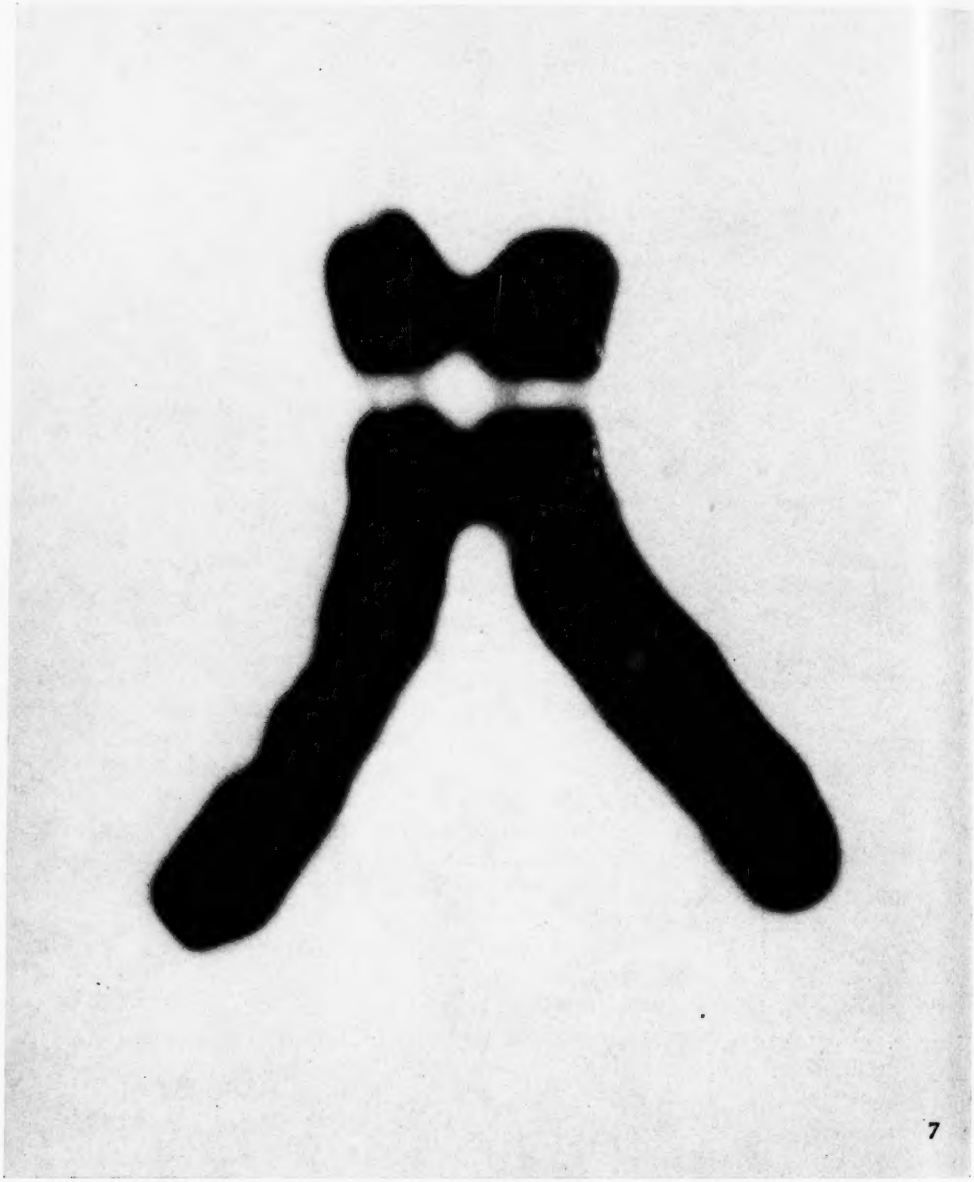


Fig. 7. *Hyacinthus orientalis.* Colchicine treated root tip chromosome at late metaphase. The two sister chromatids are held together by the most proximal regions of the arms. The kinetochores are widely separated, and in each chromatid their internal and external limits are quite clear. In this case no structure is visible at the kinetochore. — ×17800.

The structural pattern of the kinetochore can now be found to be essentially the same at the principal stages of the chromosome cycle (Figs. 8—16) and to occur among remotely related groups of plants and animals. The diagrams on the structure of the mitotic kinetochore assembled by SCHRADER (1953) and those used by ÖSTERGREN (1951) for representing the structure of the kinetochore at metaphase I of meiosis, have now only historical interest.

3. The division cycle and its implications

At prophase and metaphase of mitosis the close association of the sister chromatids remained for a long time an obstacle to an exact analysis of the division cycle of the kinetochore and of the arms. The study of the prophase and metaphase of the second division of meiosis, where this difficulty is normally absent, opened the way in this direction.

At these meiotic stages the two sister chromatids of each chromosome are, in most organisms, widely separated from each other throughout most of their length, a phenomenon that permits accurate checking of the region at which they are held together. A study of the chromosomes of *Agapanthus umbellatus* at the second division of meiosis revealed that, as early as pro-metaphase II, the two longitudinal halves of the kinetochore are distinctly separated from each other. The region of the chromosome body that holds together the two sister chromatids of each chromosome until the beginning of anaphase II is the most proximal region of the arms and not the kinetochore (LIMA-DE-FARIA, 1953). The same situation is to be found in *Fritillaria meleagris*, *Tradescantia virginiana*, *Rhoeo discolor*, *Mecostethus grossus* (Figs. 16, 41—45), and can be seen in the chromosome drawings of *Culex pipiens* (CALLAN and MONTALENTI, 1947), *Drosophila pseudoobscura* (DARLINGTON, 1934), and *Eneoptera surinamensis* (PIZA, 1946).

It was expected that this phenomenon might also be analysed at metaphase of mitosis, if, by means of a particular treatment, the sister chromatids could be separated from each other for most of their length. This is the case in materials treated with colchicine and other chemicals where X-shaped chromosomes appear. The treatment of root-tips of *Allium Cepa* with 0,01 % colchicine solution for 32 hours clearly revealed the same phenomenon (LIMA-DE-FARIA, 1955 a) and the mitotic chromosomes of *Hyacinthus orientalis* treated with 0,2 % colchicine for 8—38 hours display the same features. At c-mitotic metaphase the two sister chromatids of each chromosome are well separated from each

other throughout most of their length, being only held together at the most proximal region of the arms and not at the kinetochore (Figs. 7, 37 and 38). Colchicine and other chemical treatments disclose the same feature at metaphase of mitosis in different organisms. Although the following authors did not draw the necessary conclusions from their observations, this phenomenon was distinctly depicted after colchicine treatment in *Allium Cepa* (O'MARA, 1939, BERGER and WITKUS, 1943, WITKUS and BERGER, 1944), *Bellevalia Webbiana* (CHIARUGI, 1949), *Bupleurum tenuissimum* (GARDÉ and MALHEIROS-GARDÉ, 1949), *Galinsoga parviflora* (HASKELL and MARKS, 1952), *Habrobracon pectinophorae*, a wasp, (INABA, 1941); after iso-colchicine, sulphanilamide, and seed extract treatments in *Allium Cepa* (STEINEGGER and LEVAN, 1947, PETERS, 1946, MOTA, 1952), and after acenaphthene in *Tradescantia* sp. (SWANSON, 1940).

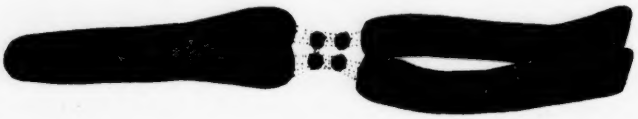
An attempt was thereafter made to study the same phenomenon under normal conditions. In chromosomes with sub-terminal kinetochore, the very short arms may have a length which equals that of the most proximal region of the long arms. This is the case in *Galtonia candicans* and *Vicia faba* where most chromosomes of the complement have one very short arm. A study of the root tip mitosis of these species (LIMA-DE-FARIA, 1955 a) revealed that at metaphase the long arms of the two sister chromatids are individualized throughout most of their length but are kept together by a weakly stained structure or substance which is more dense at the proximal regions, and the very short arms can also be seen to be quite firmly attached to each other, while the two longitudinal halves of the kinetochore are distinctly separated (Figs. 35 and 36). This is also to be observed in the figures of untreated metaphase of mitosis in *Crocus Aucheri* (MATHER, 1932), *Narcissus tazetta* (FERNANDES, 1937), *Fritillaria Raddeana* (KOSLOV, 1937), *Fritillaria latifolia major* (DARLINGTON, 1940 a), *Navicula radiosa*, a diatom (GEITLER, 1951), *Psectrocladius obvius*, a fly (BAUER and BEERMANN, 1952), where several chromosomes have one very short arm. That the kinetochore is also divided at this stage in untreated chromosomes with long arms, where the sister chromatids are normally individualized but keep in close contact throughout most of their length, may be seen in *Trillium grandiflorum* (SHARP, 1934), *Allium Cepa* (KOSLOV, 1937), *Uvularia perfoliata* (BARBER, 1941), *Rhoeo discolor* (WALTERS and GERSTEL, 1948), *Drosophila testacea* (BURLA, 1950), the mouse (TJIO and LEVAN, 1954) and *Ulophysema öresundense*, a crustacean (MELANDER, 1950). The attachment of the chromatids throughout the whole long arm is in most

materials particularly intimate at the proximal region of the arms. Especially at prophase of mitosis, before the matrix has fully developed, this differential behaviour may be seen (Fig. 34). The chemical treatments, by opening out the arms, only put in evidence the underlying difference existing among the arm regions.

After the study of this phenomenon at the second division of meiosis and at mitosis, an analysis of the first division of meiosis was required, since, due to previous difficulties in delimiting the kinetochore at this division, no consistent information was available. The analysis of the structure and division cycle of the kinetochore in *Tradescantia virginiana* at anaphase I (Fig. 39), revealed that the two longitudinal halves of the kinetochore are well separated from each other, and that the region that holds together the two sister chromatids of each chromosome is the proximal region of both the short and long arms (LIMA-DE-FARIA, 1955 d). The same situation occurs in *Tradescantia bracteata* (Figs. 12—14, 32, 33 and 40) and in *Secale cereale* (LIMA-DE-FARIA, 1955 d). It can now be seen that the structures depicted at anaphase I in: *Tradescantia reflexa* (NEBEL, 1935, SCHRADER, 1939), *Trillium Smallii* (IWATA, 1940), *Romalea microptera*, a locust (MICKEY, 1946) and *Apteromantis bolivari*, an insect (MATHEY, 1949) show the kinetochore divided at this stage, the most proximal regions of the arms being also responsible for holding together the sister chromatids. In *Allium cernuum* treated with colchicine, PRAKKEN and LEVAN (1946) observed at the same stage that the sister chromatids of each chromosome were only united by thin connections at the most proximal region of both arms, but, led by the established ideas on the division cycle of the kinetochore, they assumed these connections to be the external layers of a central body which they did not observe.

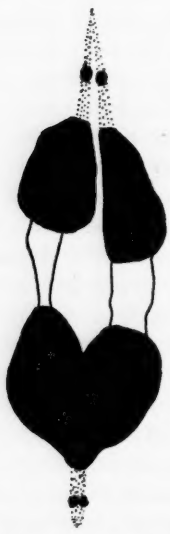
The transference to the arms of a property that until recently has been attributed by most cytologists to the kinetochore (SCHRADER, 1953, WHITE, 1954) leads to a better understanding of the following problems: (1) the relation between the kinetochore and the formation of chromosomal fibres, (2) the differentiation of the chromosome body into regions with distinct division cycles, (3) the binding process involved at the chromosome surface, and (4) the occurrence of crossing over at the kinetochore and in its vicinity.

In the cases referred to above, where the kinetochore was seen to be divided, it usually consisted of two longitudinal halves distinctly separated. There is, however, indication that at both mitosis and meiosis it is quadripartite. At mitosis, the metaphase chromosomes of untreated



8

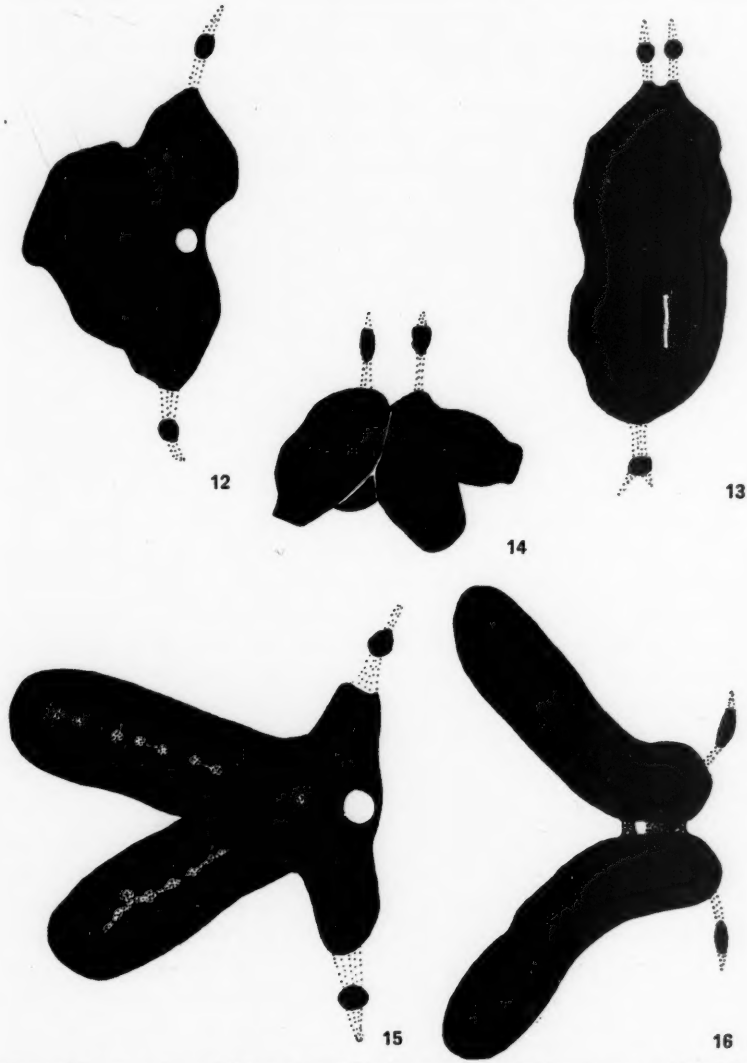
9



10

11

Figs. 8—16. The establishment of the homology of the three kinetochore zones at mitosis and at both divisions of meiosis. — Fig. 8, *Hyacinthus orientalis*, early metaphase of mitosis. Fig. 9, *Secale cereale*, pachytene (the kinetochore is at the centre of the picture). Figs. 10—11, *Tradescantia virginiana*, metaphase I. Figs. 12—13, *Tradescantia bracteata*, metaphase I. Fig. 14, *T. bracteata*, anaphase I. Figs. 15—16, *Mecostethus grossus*, metaphase I and metaphase II respectively. In the chromosomes of the last species the kinetochore is fully terminal, in the others is it median or



sub-median. The sharp folding at the interior zone of the kinetochore, which takes place at metaphase I, may be seen in Figs. 10 and 11. The division of the kinetochore starts at metaphase I (Fig. 13) and is accomplished at anaphase I (Fig. 14). The most common type of observed kinetochore structure is depicted in each case where the three zones, with a corresponding structural differentiation, may be seen. Fig. 8, colchicine treated, Figs. 9—16, no treatment. — All camera lucida drawings. — Figs. 8, 12—16 ×6500, Fig. 9 ×4500, Figs. 10—11 ×3500.

Trillium grandiflorum (SHARP, 1934) and of X-rayed and ultraviolet treated *Tradescantia* sp. (SWANSON, 1947) show four separated chromatids at the kinetochore, and at anaphase I of *Tradescantia reflexa* (NEBEL, 1935, SCHRADER, 1939) the same situation seems to occur. At the kinetochore, as in the arms, the degree of multiplication of the strands is probably dependent on the material and the stage examined. The number of functional kinetochores present at a certain stage has, however, a direct bearing on chromosome movements. The fact that, at anaphase I of meiosis, at least two distinctly separated kinetochores are present in each chromosome, not only shows that the surface of the kinetochore available for contact with the spindle is greatly increased, but also that two kinetochores may be functional at this stage, two closely juxtaposed chromosomal fibres being organized instead of one.

Whereas it is known that the whole chromosome body presents different degrees of division into strands according to different tissues and materials (*cf.* HUSKINS, 1947), it turns out now that not all chromosome regions have the same cycle of division. Within the chromosome body three main regions with distinct cycles of division are present (LIMA-DE-FARIA, 1953). At the second division of meiosis, in *Agapanthus*, the distal and median regions of the arms are divided at least since early prophase, the kinetochore divides at pro-metaphase II and the most proximal region of the arms at the beginning of anaphase II. In colchicine treated *Allium Cepa* chromosomes the behaviour of the telomeres is disclosed and besides the three regions mentioned, the chromosome ends also show a division cycle of their own (LIMA-DE-FARIA, unpublished). The chromosome body is, thus, not accomplishing its division simultaneously, this differential behaviour being exhibited by well delimited zones known for other relevant properties.

Configurations representing daughter chromosomes firmly attached at various regions of their arms have been observed in mitotic anaphases of various materials following their exposure to a wide number of chemicals and to X-rays (ÖSTERGREN, 1948, LEVAN, 1949, D'AMATO, 1949, RESENDE and MANARTE, 1951, REES, 1953 and others). Aberrant meiotic metaphase bivalents showing similar connections were described by DARLINGTON (1949) and DARLINGTON and LA COUR (1953) after mustard gas and X-ray treatments. D'AMATO (1950) suggested that mitotic pseudochiasmata (as these attachment regions are generally called), originate by a process involving the breakage and reunion of chromonemata. DARLINGTON (1949) and DARLINGTON and LA COUR (1953) interpreted both the mitotic pseudochiasmata and the meiotic

»partial chiasmata» as due to errors of chromonemata reproduction, breakage and reunion. ÖSTERGREN (1948) and LEVAN (1949) had already pointed out that pseudochiasmata occur at strictly localized points and distinguished this phenomenon from the general »stickiness» observed in other treatments as representing »point effects». RESENDE and MANARTE (1951) emphasized the role of the matrix in the formation of pseudochiasmata but the difficulties in attributing this phenomenon to a process other than the abnormal reproduction or breakage and reunion of chromonemata were principally due to our knowledge of the behaviour of the matrix being mostly derived from pathological or particular conditions following chemical and physical treatments, where a general disturbance of the matrix has been often found and described under the term stickiness. The behaviour of the most proximal regions of the arms furnishes the first example of a well defined region of the chromosome body where a localized activity of the matrix takes place in a wide number of organisms under natural conditions. The sister chromatids separate at the beginning of anaphase of mitosis and at early anaphase II of meiosis when this localized activity of the chromosome surface ceases to act. Connections between chromosomes at anaphase I involving an activity of the matrix are known under natural conditions (RESENDE and MANARTE, 1951, ÖSTERGREN and VIGFUSSON, 1953, and WALTERS, 1954). Also under natural conditions are formed the attachments between sister chromatids of B chromosomes of the grasses seen at the first pollen mitosis (*cf.* MÜNTZING, 1954). Whether pseudochiasmata, partial chiasmata and non-disjunction regions represent a process involving only the chromonemata, only the matrix or both cannot be decided at present, but the process found at the proximal regions of the arms, due to its specific characteristics and general occurrence compels us to reevaluate our interpretations of the phenomena taking place at the chromosome surface.

The fact established by the *Drosophila* workers that crossing over is less frequent in the vicinity of the kinetochore than elsewhere may now have its natural explanation. At pachytene, where crossing over takes place at the four strand stage, the individualization of the chromonemata into strands able to participate in the crossing over process can hardly occur or only occurs under particular conditions at the most proximal regions of the arms since the two sister chromatids of each chromosome keep undivided at these regions until the end of metaphase II of meiosis. At the stages during which the separation of the sister chromatids takes place, the matrix appears to constitute the only

binding element that holds together the chromatids at the most proximal regions of the arms; but at earlier stages, such as prophase II, it can be seen that also the reproduction of the chromatids is delayed at these regions (LIMA-DE-FARIA, 1953). In the case of the kinetochore, crossing over is bound to occur under similar conditions, but as the kinetochore divides earlier than the most proximal regions of the arms, it turns out that, contrary to what has been supposed before, crossing over has a bigger chance of occurring at the kinetochore than at the most proximal regions of the arms. That these last regions are undivided until metaphase of mitosis and until late prophase II of meiosis in *Drosophila* species can be seen in the figures of BURLA (1950) and DARLINGTON (1934). Both the kinetochores and the most proximal regions of the arms of a bivalent are segregated from one another at division I of meiosis and not merely the kinetochores as previously supposed (*cf.* WHITE, 1954, CATCHESIDE, 1951). Similar segregation at the first division is supposed to occur for all parts of the homologues between the kinetochore and the position of the first chiasma, but now, instead of the kinetochore, the distal part of the most proximal region of the arm should be considered as the point of reference.

4. Criteria used in the delimitation of the kinetochore

One of the main difficulties in the study of the kinetochore has been the absence of a sure criterion for its delimitation at all stages. This has resulted in the designation under the same name of quite different structures.

There are at present five main criteria of delimitation.

First, the functional criterion, which is the original one and still the most important. The kinetochore is recognized by the V-shaped appearance of two armed chromosomes in the mitotic metaphase plate and by the leading position that it takes at anaphase. Associated with the functional criterion is a morphological one. The region at which the metaphase chromosomes are bent is usually poor in DNA content and due to its sharp boundaries is a well delimited segment that has been called the primary constriction. As an immediate consequence of the establishment of this region, the relative length of the arms is obtained and it is this information which is subsequently used to indicate its position at other stages such as pachytene, diplotene, etc., where the kinetochore is not involved in active mobility on the spindle. The relative length of the arms is, however, not a sufficient source of information unless it is

Fig. 17. One of the large chromosome bivalents of *Mecostethus grossus* at metaphase I of meiosis. The kinetochores of both chromosomes are in focus and the three zones may be seen. The chromomere of the median zone is very large and distinct. No spindle is visible in these preparations. — ×13000.

Fig. 18. *Tradescantia bracteata*, bivalent at metaphase I. The three zones may be seen in the upper and lower kinetochore. The lower kinetochore is slightly out of focus. — ×14300.

accompanied by a differentiation of the arms in the vicinity of the kinetochore, which is repeated in the different chromosomes of the complement, such as the gradients at pachytene. Morever, — and here is introduced the fourth criterion — the structure observed within the region delimited in this way, needs to have a structural pattern that conforms with that observed at metaphase of mitosis within the region delimited as the kinetochore. The fifth criterion is furnished by the general occurrence of the attachment of the sister chromatids on both sides of the kinetochore which serves as a very reliable additional means of delimitation.

It is by using the four first criteria that the kinetochore has been delimited at pachytene in rye and *Agapanthus* and at diplotene in the lampbrush chromosomes of *Triturus*. The knowledge of the fifth criterion has been decisive in delimiting the kinetochore at anaphase I in *Tradescantia*. At mitosis and at the second division of meiosis the two first criteria are usually sufficient. These criteria used together now permit an accurate delimitation of the kinetochore in most organisms and at nearly all stages.

There are, nevertheless, cases where a delimitation of the kinetochore is still not feasible, such as in salivary gland chromosomes of *Drosophila* (BRIDGES, 1935), *Chironomus* (BEERMANN, 1952) and *Sciara* (CROUSE, 1943) where both a conforming structural pattern and a differentiation of the arms are missing, and the same occurs at pachytene in such species as *Lilium pardalinum* (BELLING, 1928 a), *Sorghum intrans* (GARBER, 1947) and *Trimerotropis gracilis sordida* (COLEMAN, 1948).

5. Similarities and differences between the kinetochore and the arms. The degree of heteropycnosis

As may be seen from the difficulties still present in delimiting the kinetochore at some stages, the distinction between the structure of the arms and the structure of the kinetochore is not sharp in several cases.

At present the following main structural similarities and differences may be found between the kinetochore and the chromosome arms.

Similarities:

(1) In rye pachytene chromosomes, the kinetochore was found to be composed of fibrillae and chromomeres morphologically indistinguishable from those present in the arms. Fibrilla pairs as weakly stained as those found at the exterior zone of the kinetochore occur at regular intervals in the arms, and chromomeres still smaller than those present

at the kinetochore are found at the chromosome ends (LIMA-DE-FARIA, 1952 a). In pachytene chromosomes where a similar detailed comparative study has been undertaken, such as in *Agapanthus* (LIMA-DE-FARIA, 1950, 1954), the same turned out to be true. In the lampbrush chromosomes of *Triturus* the kinetochore is also composed of fibrillae and chromomeres morphologically similar to those of the arms (GALL, 1954).

(2) At metaphase of mitosis and at metaphase I of meiosis, the kinetochore is composed of fibrillae and chromomeres. At these stages the arms do not exhibit a chromomere structure but these fibrillae and chromomeres are morphologically identical with those exhibited by the arms at other stages where their chromomere structure becomes apparent.

Differences:

(1) In the lampbrush chromosomes of *Triturus* the kinetochore chromomeres do not show the loops which are present in the chromomeres of the arms (GALL, 1954) and in maize pachytene chromosomes the fibrillae of the kinetochore seem to be thicker than those of the arms (MCCLINTOCK, 1933).

(2) The kinetochore has a condensation cycle distinct from that of the arms. Whereas most arm regions condense progressively as they approach metaphase of mitosis and metaphase I of meiosis, the kinetochore remains an uncondensed or less condensed region. From this results that the kinetochore is at these two stages negatively heteropycnotic. ÖSTERGREN (1950) has already designated as negatively heteropycnotic the kinetochore during metaphase of mitosis. His designation of the »kinetochore region» or »kinetochore» as isopycnotic at metaphase I of meiosis was, however, due to the absence of a criterion for the delimitation of the kinetochore at this stage which led him to include in this region a part of the arms (ÖSTERGREN, 1951). Now that several accurate criteria for the delimitation of the kinetochore at this stage are available, such a designation turns out to be incorrect (LIMA-DE-FARIA, 1955 d). As shown above, the kinetochore consists at metaphase and anaphase I of meiosis of fibrillae and chromomeres building a pattern which conforms with that observed at other stages, and which is of general occurrence (Figs. 10—15, 17—22, 26—33, 39 and 40). This clearly shows that the kinetochore is negatively heteropycnotic at metaphase and anaphase I of meiosis.

Whereas the kinetochore does not differ essentially from the arms in its structural organization, it exhibits a certain number of particular

features which are to be expected in a specialized region of the chromosome body.

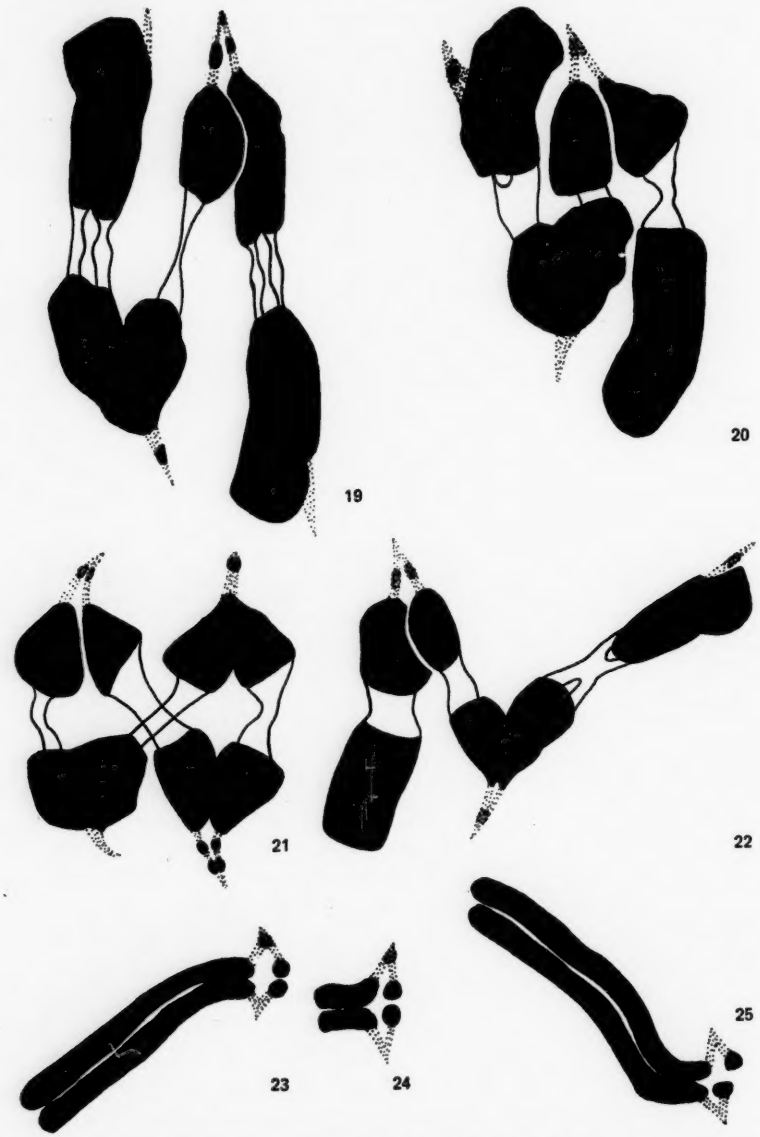
III. THE GENETICS OF THE KINETOCHORE

DARLINGTON has on several occasions referred to the kinetochore as containing centrogenes, or described it as a gene or a compound gene (DARLINGTON, 1940 a, 1947, 1949), but by making such isolated statements he has not contributed much more than others — who have ignored the issue or deliberately avoided it — to clarify the genetics of the kinetochore.

1. The kinetochore as a reversed repeat

The most striking feature of the structure of the kinetochore is its symmetric pattern. If we imagine a plane passing through the middle of the kinetochore, the latter becomes divided into two parts related one to the other as an object to its image in a plane mirror. This feature can be clearly seen in the pachytene chromosomes of rye (Figs. 9, 47 and 48), in the lampbrush chromosomes of the newt, in the mitotic chromosomes of *Hyacinthus* (Figs. 3—5), and at metaphase I of meiosis, where the folding makes it still more striking (Figs. 19—22, 26—31). In rye, for instance, where the symmetric condition is most distinct, one half of the kinetochore is composed of an almost unstained fibrilla, a chromomere and part of the centrally located deeply stained fibrilla, where minor chromomeres may be found and the other half is composed of the same components in reversed order.

The two halves of the kinetochore are not only morphologically similar but there is evidence that they are also functionally alike. A type of B chromosome called »standard» and found in rye, possesses one short and one long arm which can be structurally differentiated from each other at pachytene (Fig. 49). Two iso-chromosomes derived from »standard» have been studied in detail at this stage and shown to represent the doubling of the short and long arms respectively of the standard B (MÜNTZING and LIMA-DE-FARIA, 1949, 1953). One iso-chromosome type is apparently derived from the doubling of the short arm plus the left half of the kinetochore, the other from the doubling of the long arm plus the right half of the kinetochore (Figs. 50 and 51). Both iso-chromosomes undergo mitotic and meiotic divisions in a normal way and the structure of their kinetochores does not differ essentially from



Figs. 19—25. The folding of the kinetochore at meiosis and mitosis. — Figs. 19—22. *Tradescantia virginiana*, tetravalents at metaphase I. In one or two chromosomes of

that of the chromosome type from which they were derived (MÜNTZING and LIMA-DE-FARIA, 1953, LIMA-DE-FARIA, 1955 c). In maize (MCCLINTOCK, 1938), the two halves of the kinetochore were also found to have essentially the same properties, as a rod and a ring chromosome, having each half of the kinetochore of chromosome V, were twice obtained after X-ray treatment and participated successfully in the mitotic process.

These results allied to the general occurrence of the symmetric pattern confirm the suggestion (LIMA-DE-FARIA, 1949 b) that the normal kinetochore is essentially a tandem reversed repeat.

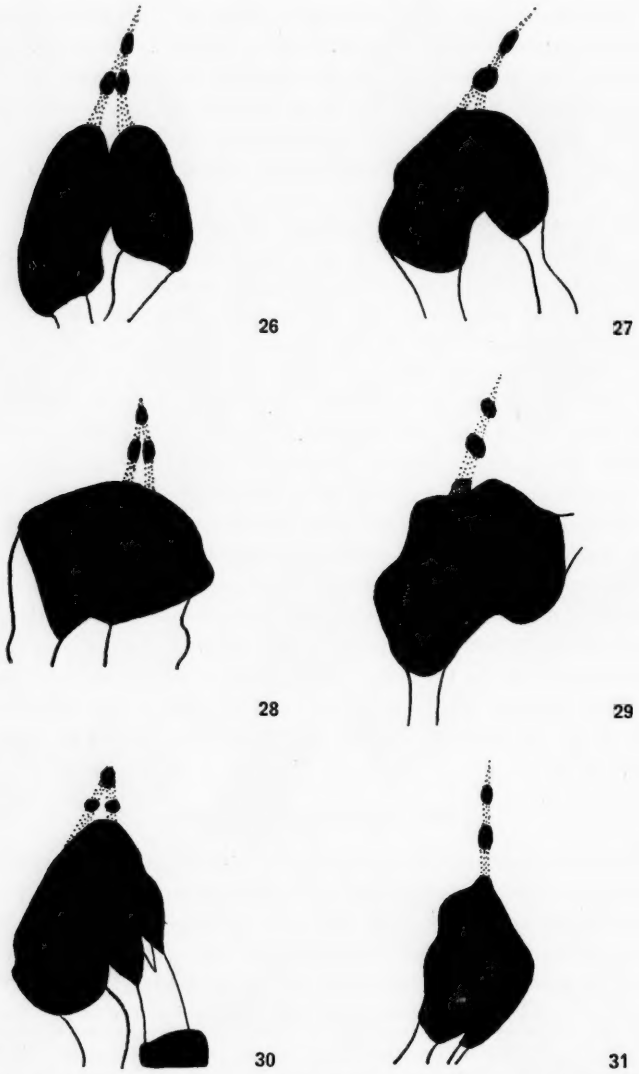
It is, thus, not surprising that telocentrics resulting from breakage through the interior zone of the kinetochore can not only undergo several cell generations but also easily give rise to iso-chromosomes which have no difficulty in surviving, since the kinetochore pattern thus formed is essentially like the original one. The abundance of cases of formation of iso-chromosomes described in cytological literature is easily explained in view of this property of the kinetochore. The survival and stabilization of iso-chromosomes could hardly be conceived if the normal kinetochore were not a reversed repeat.

This feature of the kinetochore is also in agreement with its division of the chromosome body, in most organisms, into two arms whose properties bear a relation to its location. Of particular significance is the finding that all of the tandem repeats, which have been described for the normal salivary gland chromosomes of *Drosophila melanogaster* and in which the sequence can be determined, are of the reversed type (LEWIS, 1945).

2. The induction of breakage

Misdivision (DARLINGTON, 1939) is a misleading term, — although a short, well-sounding word — since it contains an implication which is not necessarily correct. By the use of a particular word for the occurrence of breakage at the kinetochore one includes in a separate category breakage events which cannot be recognized as distinct from those occurring in the chromosome arms. The term was coined when

each figure the folding of the kinetochore can be followed accurately due to the slight separation of the two chromosome arms (Figs. 19 and 20 upper right, Fig. 21 upper left and lower right, and Fig. 22 upper left). — Figs. 23—25. Chromosomes of *Galtonia candicans* at late metaphase of mitosis. The folding is very distinct here as the short and long arms normally keep far apart. The chromomere of the median zone is seen in Figs. 23 and 24. — All camera lucida drawings. — Figs. 19—21 ×3150. Fig. 22 ×2250. Figs. 23—25 ×4000.



Figs. 26—31. Compound structure of the kinetochore at metaphase I of meiosis in *Tradescantia virginiana*. One of the chromosomes of a bivalent or multivalent is shown in each figure. The two chromomeres of the median zone and one at the interior zone may be seen in most figures. The chromomere of the interior zone is probably compound in most cases as the kinetochore is folded; this can be seen in

the phenomenon was studied adequately for the first time (DARLINGTON, 1939) and it is thus not surprising that it should arouse such an interpretation. Today it can, however, be seen that this interpretation no longer fits within the framework of our knowledge. The description of the phenomenon was based mostly on the observation of transverse division at the kinetochore occurring under disturbed meiotic conditions, such as in univalents at metaphase I and daughter univalents at metaphase II. Transverse division is known to take place as well in the arms during disturbed meiotic conditions, such as in bivalents showing difficulties of separation or in chromatid bridges; and moreover, we know today that kinetochore breakage can be induced by physical or chemical agents just as in the arms.

Breakage events occurring at the kinetochore can be classified into four categories according to their main agent: (1) appearing under disturbed meiotic conditions, (2) originating after the action of X-rays, (3) produced by chemical treatment, and (4) occurring spontaneously.

The first group constitutes the main bulk of the evidence since meiotic disturbances have been extensively studied. Breakage at the kinetochore was observed at meiosis in: monosomic wheat plants (SEARS, 1944, 1952, UNRAU, 1950, MORRISON and UNRAU, 1952, MORRISON, 1953 a), speltoid and subcompactoid wheat (SANCHEZ-MONGE and MAC KEY, 1948, SANCHEZ-MONGE, 1951), other wheat strains showing various degrees of aneuploidy (FRANKEL, 1949, MORRISON, 1953 b), *Godetia* and rye haploids (HÅKANSSON, 1940, LEVAN, 1942), maize and barley trisomics (RHOADES, 1936, 1938, 1940, SMITH, 1941), a rye univalent introduced into *Triticum vulgare* (O'MARA, 1951), B chromosomes of rye (MÜNTZING, 1944), univalents arising in *Fritillaria* and *Chrysanthemum* (DARLINGTON, 1939, DOWRICK, 1952), *Campanula* plants showing rings and chains (DARLINGTON and LA COUR, 1950), and other cases. It is of importance to note that in all these cases breakage at the kinetochore is preceeded by the formation of univalents and it occurs practically only in this chromosome type.

SANCHEZ-MONGE (1950 a and b) suggested that kinetochore breakage may be due to an action of the spindle poles acting on the kinetochore components in opposite directions, but at that time the structure and orientation of the two kinetochore halves at metaphase and anaphase I

Fig. 21 lower right. Surmounting this chromomere is a third short fibrilla, which shows that the chromomeres of the interior zone are separated a certain distance apart (see also Fig. 21). No spindle is visible in these preparations. — All camera lucida drawings. — ×4500.

was unknown. The formation of univalents creates a condition to which the kinetochore is not adapted, since its orientation in the spindle at metaphase I is normally made in connection with a chromosome partner. At anaphase I in a chromosome that belonged previously to a bivalent, the two kinetochores — each corresponding to a sister chromatid — are both folded and orientated towards the same spindle pole (Figs. 14, 39 and 40). In such a chromosome the left and right halves of each kinetochore are both orientated to the same pole (LIMA-DE-FARIA, 1955 d). In a univalent that lies somewhere in the cell or at the equator these conditions become difficult of fulfilment and as both kinetochore halves can function independently, as shown above, the left half of the kinetochore may become orientated to one spindle pole and the right half to the other spindle pole, and breakage results. The situation met with here is similar to that found in a common dicentric where both kinetochores function independently of each other, and may separate to opposite poles. In a univalent this process arises just within the kinetochore as both halves are able to function independently. Whether breakage affects the two sister chromatids or only one, may depend on the degree of accomplishment of the division of the kinetochore which was found to take place during metaphase I (LIMA-DE-FARIA, 1955 d).

Kinetochore breakage following X-ray treatment was reported incidently in maize (MCCLINTOCK, 1938), in wheat (SMITH, 1947), in *Tradescantia* (HAQUE, 1953), where the formation of telocentrics was described, but recently GOTTSCHALK (1951 a and b) demonstrated clearly this effect. After X-ray treatment of inflorescences of *Solanum lycopersicum* 73,2 % of all break points occurred at the kinetochore, whereas only 18,4 % and 8,4 % of the break points were located in the hetero- and euchromatin respectively.

KARPECHENKO (1940) described kinetochore breakage in barley root tips after colchicine treatment, CARPENTIER and PACAULT (1949) found it after the action of benzene on the roots of *Allium Cepa* and KOLLER (1947) and DARLINGTON and KOLLER (1947) also observed it after exposure to mustard gas of inflorescences of *Tradescantia*. The first chemicals, however, that gave decisive evidence of a direct action on the kinetochore were urethan and aluminium chloride. A comparative study of the breakage frequency at the kinetochore and at the heterochromatic and euchromatic regions of the pachytene chromosomes of *Solanum lycopersicum*, after treatment of inflorescences with these chemicals, revealed that in a total of 1463 break points, breakage at the kinetochore

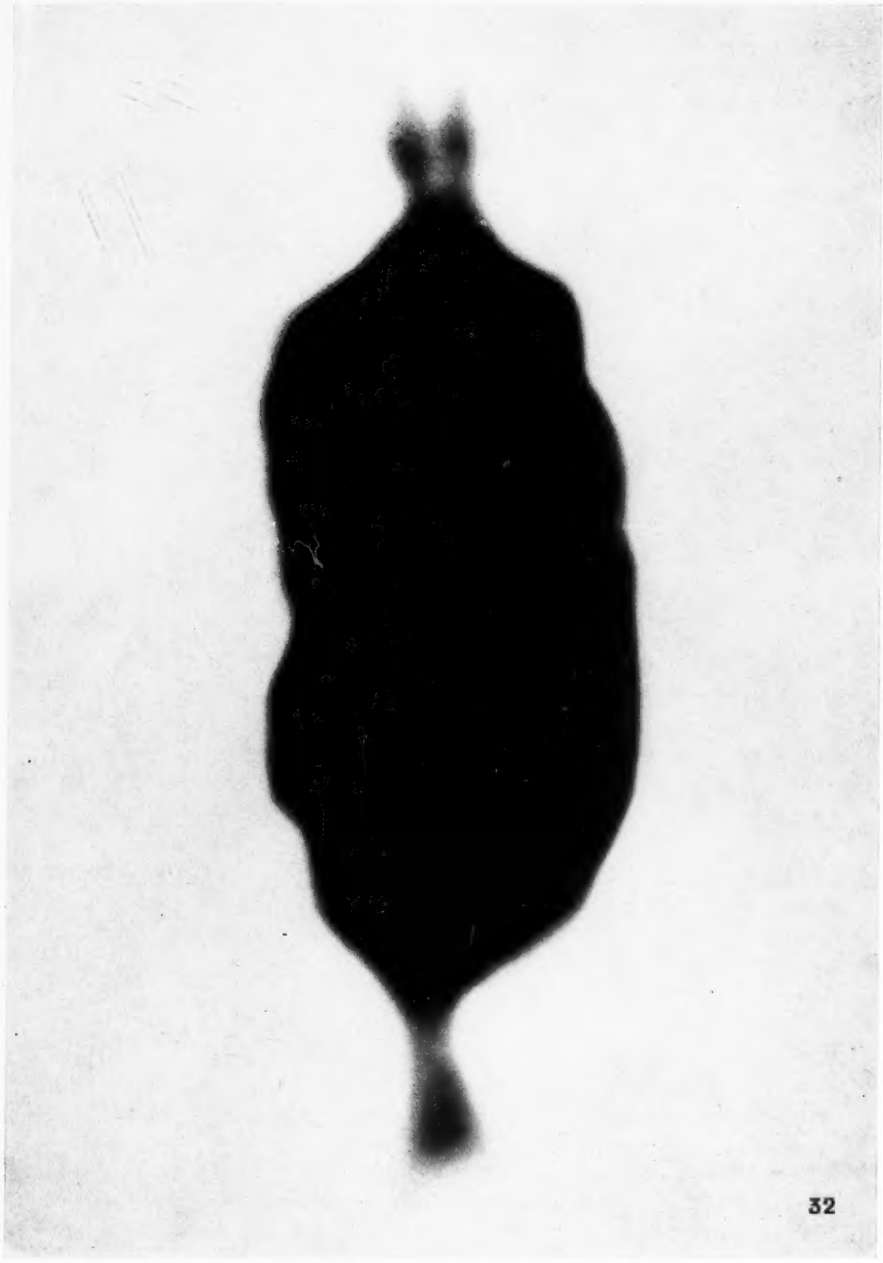


Fig. 32. The kinetochore starting to divide at metaphase I in a bivalent of *Tradescantia bracteata*. The upper kinetochore is already divided exhibiting the three zones distinctly. The lower kinetochore has not accomplished its division to the same extent, but it shows already a thickness which is approximately equal to that of both sister kinetochores of the upper chromosome. Kinetochore and arms stained by the Feulgen test. Compare with the camera lucida drawing in Fig. 13. — ×16000.

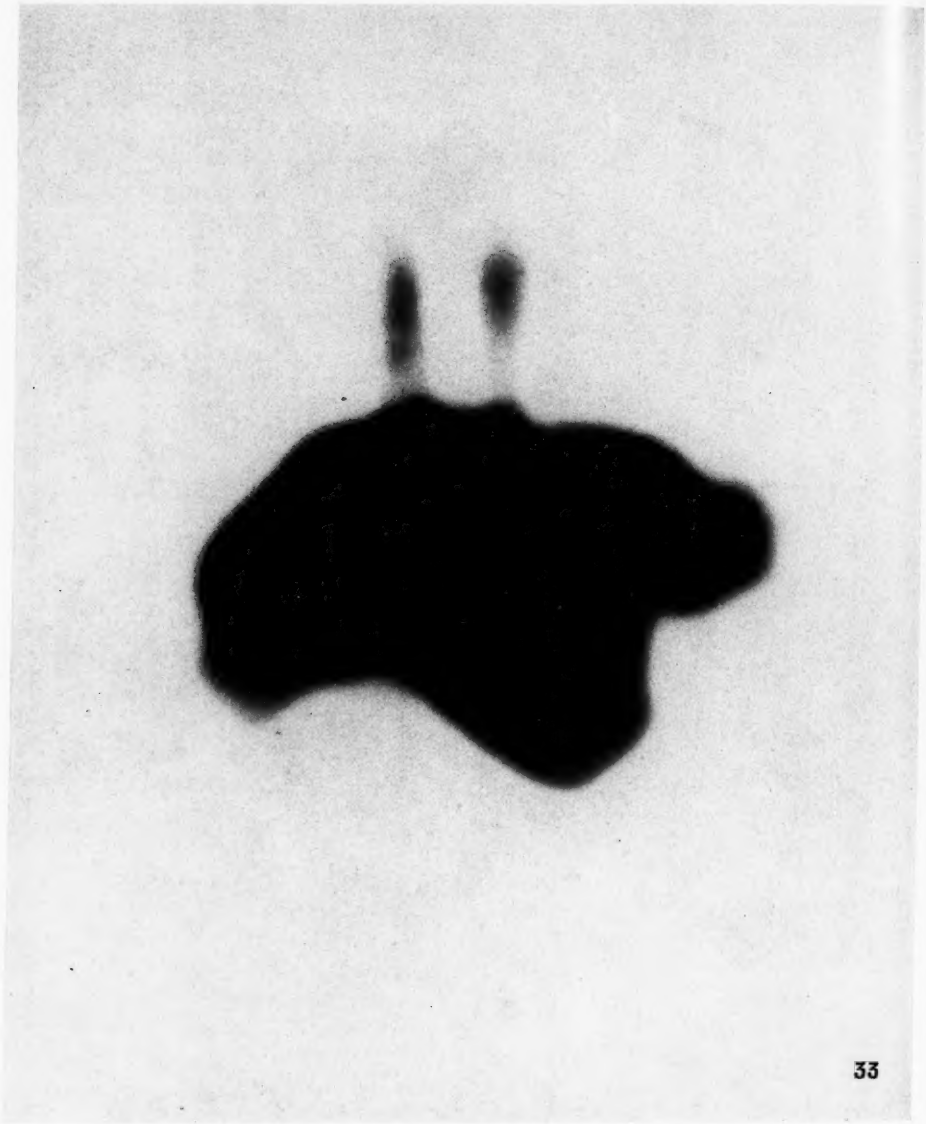


Fig. 33. Chromosome of *Tradescantia bracteata* at anaphase I showing the kinetochores (each belonging to a chromatid) separated far apart and parallel. Right kinetochore slightly out of focus. Compare with Fig. 14. — ×13600.

occurred in 80,4 % of the cases whereas at the two other regions only in 8,0 % and 11,6 % respectively (GOTTSCHALK, 1951 b). The nucleolar constriction and the kinetochore of the nucleolar chromosome of *Vicia faba* also turned out to be especially liable to breakage after chemical treatment (GLÄSS and WOLL, in OEHLKERS, 1953). In the experiments with heavy metals, breaks in the nucleolar constriction are twice as frequent as in the kinetochore, while on the other hand nucleic acids cause twice as many breaks in the kinetochore as in the nucleolar zone.

Spontaneous kinetochore breakage has been described occasionally, such as in *Nothoscordum* (LEVAN and EMSWELLER, 1938) or in *Tradescantia* (PROPACH, 1940), but a careful study of the nucleolar chromosome of *Vicia faba* has shown that spontaneous breakage is particularly high at the kinetochore and at the nucleolar zone, when compared to its occurrence at other regions of the arms (WOLL and GLÄSS, in OEHLKERS, 1953).

Breakage events at the kinetochore cannot be seen to differ from breakage in the arms. The kinetochore can exhibit a higher degree of spontaneous breakage than other arm regions and reacts to X-rays and various chemicals in a similar way. This effect is partly specific since the kinetochore and other specialized regions of the arms exhibit a differential reaction following the treatment.

3. Structural and functional variation

The frequent occurrence of breakage at the kinetochore raised the problem of the formation of structural rearrangements within this region. DARLINGTON (1940 a) was the first to visualize the consequences of this event. He assumed that breakage may take place anywhere within the kinetochore. This view was later extended in connection with the structural differentiation found within this region (LIMA-DE-FARIA, 1955 c).

The evidence available at present suggests that breakage may take place in the three kinetochore zones. In maize (MCCLINTOCK, 1938) each of the two chromosome derivatives obtained from chromosome V possessed half of the original kinetochore, which indicates that breakage took place at the interior zone. In *Tradescantia* (PROPACH, 1940) and in *Phleum* (ELLERSTRÖM and TJIO, 1950) breakage seems to have occurred at the median zone, since the break point is apparently just at one of the chromomeres composing this zone. And in maize (RHOADES, 1936, Fig. 4) a telocentric was obtained having a kinetochore of the same size

as the kinetochore from which it was derived, which strongly indicates that breakage occurred in this case at the exterior zone.

As the kinetochore is a reversed repeat, one may expect that products resulting from breakage at the interior and exterior zones will be favoured by natural selection since they tend to approach more than others the normal pattern. But the presence of kinetochores with different structures and functions reveals that the various structural elements that constitute the kinetochore may combine in a less rigid way.

Kinetochores do not show their most detailed structure in all kinds of species and not in all chromosome types. Their structural appearance bears a relation to chromosome size following apparently two rules.

(1) *The species with the largest chromosomes show the most clear structure of the kinetochore.* In their extensive study of the structure of the kinetochore at mitosis in 41 species, TJIO and LEVAN (1950 a and b) found the kinetochore chromomeres to be most clear in *Scilla sibirica* known for its large chromosomes but already smaller in *Allium* and *Hordeum.* In species with small chromosomes they were unable to detect any kinetochore chromomeres. The reason for this difference is not to be sought in a degree of chromosome contraction, as assumed by these authors, but in the size of the kinetochore. In the small chromosomes, such as those of *Vicia cracca*, a whole chromosome including its two arms and kinetochore measures one and a half micron just as much as the whole kinetochore of the chromosomes of *Scilla sibirica* at the same stage and after the same treatment. The kinetochore in the chromosomes of this *Vicia* species and in other species with small chromosomes has at mitosis a size which is five to eight times smaller than that of *Scilla* and *Allium* chromosomes. Of the circa 40 species referred to above in the section dealing with the structure of the kinetochore pattern, all of them, except one or two, are known for their particularly large or relatively large chromosomes, the Liliaceae are heavily represented and next come several species of *Tradescantia.* These are the species where the kinetochore structure turned out to be most distinct in a selection made from practically all the cases found in the literature in which the kinetochore has been depicted. Due to the extent of the observations made, this relation can hardly be considered to be fortuitous.

(2) *Within a chromosome complement the largest chromosomes tend to exhibit the clearest kinetochore structure.*

In *Vicia faba* (n=6) five of the chromosomes of the complement are of about the same length but the sixth chromosome, the nucleolar chro-

34

35

37

36

38

Figs. 34—38. The division cycle of the kinetochore at mitosis. — Figs. 34—35, *Galtonia candicans*. Fig. 36, *Vicia faba*. Fig. 37, *Hyacinthus orientalis*. Fig. 38, *Allium Cepa*. — Figs. 34—36, untreated prophase (Fig. 34) and metaphase chromosomes (Figs. 35—36). — Figs. 37—38, colchicine treated chromosomes at metaphase. In each case the kinetochore is divided and the most proximal regions of the arms are holding together the sister chromatids. With the exception of Figs. 39—40, no structural differentiation is visible within the kinetochore in Figs. 34—44. — All camera lucida drawings. — ×6500.

mosome. has approximately double the size of the other chromosome type. TJIO and LEVAN (1950 b), searching for the structure of the kinetochore in the chromosomes of this species, could not find it in the short chromosome type but it appeared distinctly in the large chromosome type which is five times less frequent. In the mouse, TJIO and LEVAN (1954) noticed that the kinetochore structure is more easily brought about in certain chromosomes, the kinetochore chromomeres being present quite regularly in the »A» chromosome which is one of the largest of the complement. In *Mecostethus grossus* (LIMA-DE-FARIA, unpublished) the structure of the kinetochore may be seen in most of the chromosomes of the complement, but the two largest chromosomes differ sharply from the others in the clearness with which this structure is brought about in them.

In all these cases, where the large size of the chromosomes of a species or within a complement is accompanied by a more distinct display of the kinetochore structure, the difference cannot be easily attributed to the load of the large arms, since this structure is observed most clearly at pachytene, diplotene (in lampbrush chromosomes), and prophase of mitosis, where no active chromosome movements are involved. The underlying cause is to be sought in the presence of a larger kinetochore in these chromosomes.

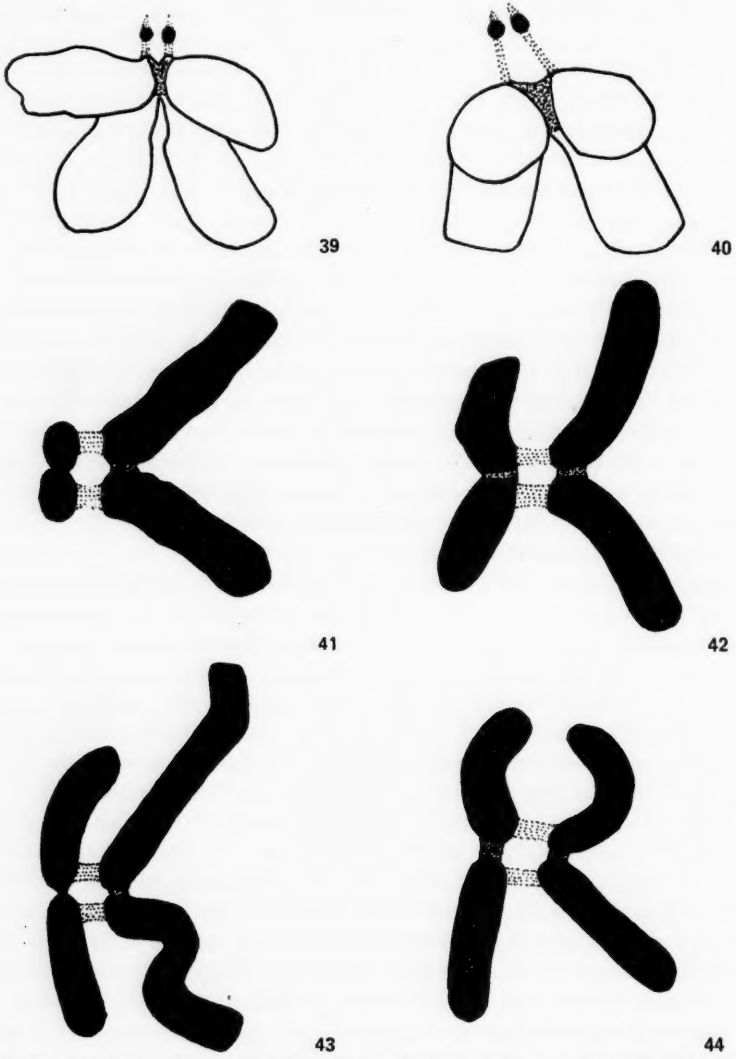
Chromosomes which within a complement show a distinctly larger kinetochore are known in several species such as *Trifolium pratense* (LEVAN, 1940), *Poa alpina* (MÜNTZING, 1948 b), *Trillium undulatum* (BAILEY, 1954), *Tulbaghia violacea* (WHITAKER and FLORY, 1955) but in these cases it is not known whether the larger size is due to an intimate connection with the nucleolar organizing region or to the kinetochore being at the same time a nucleolar organizer, as is for instance the case in *Campanula persicifolia* (LA COUR, 1950) and in *Mycetobia pallipes* (WOLF, 1950). In *Antirrhinum majus* this difficulty is, however, removed. ERNST (1939) was able to identify at pachytene the eight chromosomes of the haploid complement of this species and found chromosome VI to be characterized by a kinetochore much larger than the others. The difference is so sharp that the size of the kinetochore was used as the most important marker to distinguish this chromosome from four others which have about the same length. Chromosome VI is not associated with the building of the nucleolus. In *Allium sphaerocephalum* one of the chromosomes of the complement was also found to have a particularly large kinetochore (TJIO and LEVAN, 1950 b) and the characteristic kinetochore structure was found within its boundaries.

In different species the kinetochore structure is not necessarily alike in its details. At pachytene, in maize, rye and *Agapanthus*, the structure of the kinetochore is different. In maize the kinetochore fibrillae seem to be thicker than in rye and no chromomeres have so far been observed at the kinetochore. In *Agapanthus* some of the pachytene chromosomes show at the kinetochore two chromomere pairs of different size, which results in a characteristic egg-shaped appearance of its pattern (LIMA-DE-FARIA, 1955 c).

Not only structural differentiation but also functional variation occurs at the kinetochore. Sex chromosomes, B chromosomes, and other chromosome categories furnish many examples of differential chromosome behaviour on the spindle, which has been attributed to differences in the function of their kinetochores, or which suggests such a differential function. These cases are, however, too many and sometimes too complex for their treatment to be included in this study. Certain aspects of pairing, the division cycle of the most proximal regions of the arms and other factors need to be carefully examined before this differential behaviour on the spindle can be attributed with certainty to a differential function of the kinetochore itself.

In rye (LIMA-DE-FARIA, 1955 b) a small chromosome derivative with a deleted kinetochore has essentially the same ability to move to the spindle poles as a chromosome with a normal kinetochore, since it is not eliminated during the mitotic divisions and since it divides regularly at the first division of meiosis, elimination and non-disjunction seldom occurring (Fig. 52). It also occupies in the mitotic metaphase plate a central position similar to that of other small chromosomes with normal kinetochore. On the other hand, its behaviour differs from that of chromosomes with a complete kinetochore in that it suffers very little elimination at the second division of meiosis. Usually, rye univalents with a normal kinetochore, which divide at the first division, lag and are eliminated to a large extent at the second division. A parallel case is found in *Trillium erectum* (SPARROW, POND and SPARROW, 1952) where also small chromosome derivatives behave normally on the spindle and suffer little elimination at the second division of meiosis, although a large percentage of them are unpaired at first meiotic m aphase and divide at this stage.

In wheat (SEARS and CAMARA, 1950, 1952, STEINITZ-SEARS nd SEARS, 1953), a dicentric chromosome was found to consist of a long arm, one kinetochore, a short interkinetochoric region, a second kinetochore and a minor arm. At metaphase I, the dicentric usually behaves as a »bi-



Figs. 39—44. The division cycle of the kinetochore at meiosis. — Figs. 39—40, anaphase I chromosomes in *Tradescantia virginiana* and *T. bracteata* respectively. — Figs. 41—44, metaphase II chromosomes of *Fritillaria meleagris*, *Agapanthus umbellatus*, *Tradescantia virginiana* and *Rhoeo discolor* respectively. In each case the two sister chromatids are only in contact at the most proximal regions of the short and long arms. Figs. 39—44, no treatment. — All camera lucida drawings. — Figs. 39, 41, 43 ×4500, Figs. 40, 42, 44 ×6500.

valent» with the two kinetochores opposing each other. At this stage it lies off the plate, and is commonly included entire in one telophase group, since the sub-terminal kinetochore is functionally weaker than the other. When the dicentric pairs with another chromosome only one of its kinetochores is active, as a rule, and this is almost always the centrally located kinetochore.

As far as our methods of analysis go, these cases furnish clear evidence of functional differentiation among kinetochores.

4. Chemical composition

The introduction by HEITZ (1936) of the FEULGEN and ROSSENBECK reaction as a chromosomal staining, allied to the methods of squash introduced by BELLING, made it easy to obtain a sharp staining accompanied by a good spreading of the chromosomes. The advantages of this technique resulted in its general use as a chromosomal staining procedure, but at the same time led many cytologists to draw hasty conclusions about the chemical nature of the various chromosome regions. The staining or unstaining of a cell or chromosome region by fuchsin-sulphurous acid after hydrolysis is not in itself a proof or disproof of the presence of DNA in such a region. At the same time during the thirties many authors were not looking for a structure within the kinetochore and the finding of the »primary constriction» as an unstained or less stained zone of the chromosome body led them to describe it as »anucleal» or Feulgen negative (SCHAEDE, 1936, 1937). SCHAEDE was looking for differences in the stainability of the heterochromatin relative to the euchromatin, and only pointed out the sharp difference in stainability between the kinetochore and the arms at mitosis. The work of many other authors falls within this category of observations. It is beyond doubt that the kinetochore at metaphase of mitosis is a region poorer than the arms in DNA content, but such a situation does not permit us to draw the conclusion that it is Feulgen negative or anucleal, two designations which still persist in cytological literature.

Following this line of thought SCHRADER (1944, 1953) did not distinguish between results based on old-fashioned techniques and results obtained with modern ones. He was led to suggest that there may be a difference in DNA content between animal and plant kinetochores and that the small size of this chromosome region might be an obstacle to obtaining decisive information about its chemical constitution. The first

suggestion is based on four papers (METZNER, 1894, WILSON, 1931, SCHRADER, 1936 and BENOIT and KEHL, 1939), METZNER's work dating from the early days of chromosome studies. The technique used in the three first papers is essentially the same. Chondriosomal and other stains are used followed by a strong extraction of some of the dyes as a process of differentiation. BENOIT and KEHL used also a chondriosomal stain and their figures do not permit us to draw any conclusion about the structure stained. The regions which METZNER, WILSON and SCHRADER observed to be strongly stained by chondriosomal stains cannot be homologized with certainty with the kinetochore and are most probably the proximal regions of the arms. In any case, the affinity of a region for chondriosomal stains is not pertinent information of its DNA content. The second suggestion made by SCHRADER is also based on the studies made during the thirties when the descriptions of the kinetochore were mostly limited to a few of its structural components or to stages where this chromosome region is particularly small. These two suggestions can now be shown to be unjustified.

COLEMAN (1940), PROPACH (1940) and IWATA (1940) who studied the kinetochore more carefully already found indications that its components were Feulgen positive. In the zygotene and pachytene chromosomes of *Veltheimia viridifolia* (a Liliaceae) the kinetochore fibrillae appeared unstained, but the chromomere found between them became stained with fuchsin-sulphurous acid (COLEMAN, 1940). In most of the metaphase chromosomes of *Tradescantia gigantea* at the first pollen mitosis, PROPACH found the kinetochore to consist of a chromomere and fibrillae which become deeply coloured by the same stain. In *Trillium Smallii*, IWATA described the kinetochore of anaphase I chromosomes as composed of a fibrilla and a spherule which were also stained by fuchsin-sulphurous acid.

These were the first results strongly indicating that in plants the kinetochore contained DNA as well as the arms, but they did not furnish decisive information on this point because these authors did not apply the Feulgen test. Fuchsin-sulphurous acid is not by itself a specific reagent of desoxyribose nucleic acid, since it can form a dye with substances present in the cell like some polysaccharides, lipids, etc. (BAUER, 1933). One can only speak of the Feulgen reaction as a test for desoxyribose nucleic acid when an unhydrolysed control is made. If the unhydrolysed control, after immersion in fuchsin-sulphurous acid, shows the formation of a dye at the body in question, we are in the presence of a substance which is not desoxyribose nucleic acid and

Fig. 45. Chromosome of *Mecostethus grossus* at metaphase II of meiosis. The kinetochore is terminal. As in the cases of median (Fig. 44), sub-median (Figs. 37—40, 42, 43) and sub-terminal (Figs. 34—36, 41) kinetochores, the most proximal regions of the arms are also here holding the sister chromatids together, and the kinetochores lie free in this case. Compare with Fig. 16. — ×13000.

Fig. 46. Chromosome IV of *Secale cereale* at pachytene (a bivalent). The kinetochore is at the centre of the picture with two chromomere pairs. The gradients in chromomere size and thickness and stainability of the fibrillae, which originate on both sides of the kinetochore, may be seen. Camera lucida drawing on Fig. 9. — ×7000.

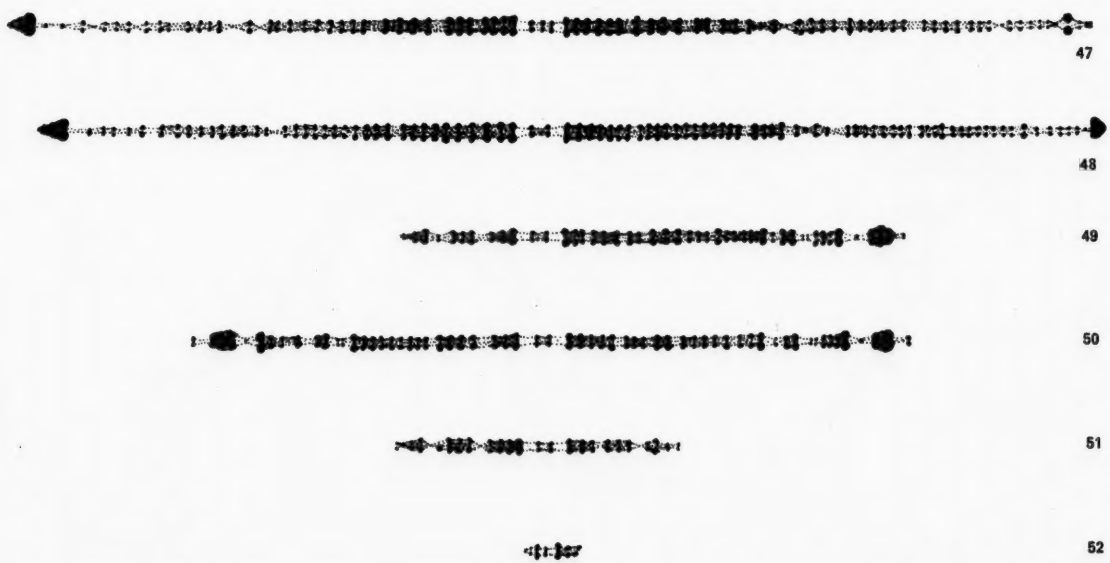
which may be, for instance, a polysaccharide; if on the contrary the undhydrolysed control does not show the formation of any dye and the hydrolysed material develops the colour characteristic of the »regenerated basic fuchsin», it is considered that it reveals the presence of DNA.

Demonstration that the kinetochore contained DNA, was obtained when the Feulgen test was applied to the pachytene chromosomes of *Agapanthus umbellatus* (LIMA-DE-FARIA, 1950). Here the precautions necessary to ensure a perfect reaction were taken and an unhydrolysed control was used. Also, in this material the limits of the kinetochore could be defined with accuracy. The whole kinetochore showed a positive Feulgen reaction, both its chromomeres and fibrillae staining distinctly. The kinetochore chromomeres stained as deeply as the chromomeres of the median and distal regions of the arms which have a similar size.

At metaphase I of meiosis, where the kinetochore is involved in chromosome movements with the participation of the spindle, it is of importance to know whether it also contains DNA. I have now applied the Feulgen test to the metaphase and anaphase I chromosomes of *Tradescantia virginiana* and *T. bracteata*, using the same precautions as before, and a strong positive Feulgen reaction develops in the three zones of the kinetochore, the fibrillae and the chromomeres of the median and interior zones staining intensely (Figs. 10—14, 18—22, 26—33, and 40).

Confirmation that the kinetochore of animal chromosomes also contains DNA was obtained by GALL (1954) in the chromosomes of the newt. In the lampbrush chromosomes from oocyte nuclei of *Triturus viridescens* the kinetochore is a well defined region of about 10 micra in length by 2 in width composed of large chromomeres. After the Feulgen test the kinetochore is stained a distinct purplish colour; due to its larger size, it appears somewhat darker than the average chromomeres of the arms.

In *A. umbellatus* the kinetochore has an average size of 2 micra in length by one in width (*cf.* LIMA-DE-FARIA, 1950, 1954). This is a size that is nearly double that of the short arms of the chromosomes of many plant and animal species at metaphase of mitosis. In *Galtonia candicans* the short arms of most of the chromosomes have at this stage an average size of $0{,}5 \times 0{,}5$ micra, and those of most chromosomes of *Vicia faba* an average size of $0{,}8 \times 0{,}5$ micra. There is no difficulty in getting an equally well defined and intense DNA reaction in these short arms. The examples could be multiplied. In *Tradescantia virginiana* at metaphase I



Figs. 47—52. Structural variability of the kinetochore in *Secale cereale*. — Fig. 47, chromosome III exhibiting a chromomere pair at the interior zone. Fig. 48, chromosome II showing the common type of structure observed at the kinetochore with a deeply stained fibrilla pair at the interior zone. Fig. 49, »standard» B chromosome type with the same kinetochore pattern. Figs. 50 and 51, the two iso-chromosomes derived from the long and short arm of this B chromosome type, exhibiting a similar structure at the kinetochore. Fig. 52, a chromosome derivative with a deleted kinetochore. — ×2150.

each folded half of the kinetochore has an average length of 2,5 micra (with the technique here used), the total kinetochore length being circa five micra. But the large size of the kinetochore of the lampbrush chromosomes of *Triturus* obviates any objections concerning the dimensions of the kinetochore in connection with the Feulgen test, for its size of 10×2 micra is of the same magnitude as that of whole long arms of the mitotic chromosomes of *Allium Cepa*, *Tradescantia virginiana* and other species known for their large chromosomes.

The existence of DNA in both plant and animal kinetochores is particularly relevant to the genetics of the kinetochore due to the role attributed to nucleic acids in gene structure and gene reproduction (CASPERSSON, 1950, STERN, 1952, WATSON, 1955).

5. Limitations of the methods used in the location of genes

Genes have been located in the chromosomes of *Drosophila* mainly by the use of two methods: (1) by means of crossover values, and (2) by use of deficiencies and other structural rearrangements occurring naturally or caused by physical or chemical agents. Crossover values only permit the seriation of genes along the chromosome body and only furnish us with indications of their relative distances. This last source of information is dependent on factors affecting the amount of crossing over per unit physical length and for this reason the crossing over method by itself does not permit the assignment of the position of a gene to a definite chromosome segment. By this method it has not been possible to locate genes at the kinetochore or at any other small and sharply delimited region of the chromosome arms.

By means of the second method genes could be ascribed to very small definite segments of the chromosome body but due to the following difficulties this method also showed itself incapable of revealing the presence or absence of genes at the kinetochore, a fact not realized by most cytologists. First, the kinetochore is a region which cannot be delimited with accuracy in salivary gland chromosomes for it is not known whether the chromocentre in *Drosophila melanogaster* represents only the kinetochores or these regions plus the most proximal regions of the arms. Secondly, the undifferentiated appearance of the chromocentre made the analysis, carried out with much precision in the arms, very difficult at this region. Thirdly, but most important, the deletion of a segment belonging to the kinetochore is not necessarily detected by this method as easily as a deletion taking place in the arms. A deletion

or a mutation arising at the kinetochore may result in a chromosome being lost and thus not being recovered in the progeny, or else, if it affects only the function of the kinetochore in a noticeable way, it may not necessarily affect the body characters which are those mainly studied by the classic progeny analysis.

Two examples clearly demonstrate this situation. The gene bobbed is the most proximally located gene in the X chromosome of *D. melanogaster* referred by BRIDGES and BREHME (1944) in their compilation of the mutants of *Drosophila*. Bobbed is given in the linkage maps a locus at 66,0 and the same value is given for the kinetochore (BRIDGES, 1935, chromosome maps, BRIDGES and BREHME, 1944, Plate 2). The same gene is located far from the kinetochore in the large proximal heterochromatic segment in the mitotic chromosome map of the X chromosome (DARLINGTON and MATHER, 1949, Fig. 9). This discrepancy of location is due to the reasons just mentioned and to the differential condensation of the heterochromatin in mitotic and salivary gland chromosomes. This large heterochromatic segment is hardly detectable in the linkage map, and is only represented by a short segment in the polytene chromosomes.

AUERBACH (1947) after treatment of wild type males of *D. melanogaster* with a mustard gas compound, observed an abnormal and inherited type of segregation on the X chromosome. The treated X was characterized by four peculiarities: it carried a semilethal, it carried a sterility mutation, it had a tendency to follow the untreated X chromosome into the same cell at meiosis (non-disjunction), and it had a still greater tendency to be lost on the spindle at meiosis. According to AUERBACH, the viability and the sterility mutation were possibly identical, having been located in the same region as carried the cause of the abnormal segregation, for all peculiarities were lost simultaneously from one female. This region appears to have been the kinetochore. The examination of the salivary gland chromosomes revealed an abnormality in the region of the chromocentre but its nature could not be determined. In this case the inherited change resulting in the tendency for non-disjunction and in the abnormal behaviour on the spindle was accompanied by other changes recognizable at the organism level, but the lack of a criterion for delimiting the kinetochore and the undifferentiated structure of the chromocentre did not permit its exact location at the kinetochore.

In maize the delimitation of the kinetochore does not offer difficulties at pachytene. However, due to the relatively small number of genes known per chromosome, the position of the kinetochore in the linkage

maps has only been tentatively established in most chromosomes (RANDOLPH, 1941, ANDERSON and RANDOLPH, 1945, RHOADES, 1950) and also, as no genes seem to have been found which have at the same time affected the behaviour of the kinetochore and the phenotype of the plant, the genetic work so far carried out has not yet borne results concerning the location of genes at the kinetochore.

In *Neurospora* the measure of the distance of a gene locus from the kinetochore is given by the recombination percentage, or half the percentage of second-division segregation obtained by the tetrad analysis (*cf.* CATCHESIDE, 1951). A gene that has a zero per cent second-division segregation is supposed to be located at or quite close to the kinetochore. One such gene, »scumbo», is known in *Neurospora crassa* (REGNERY, 1944, CATCHESIDE, 1951). As here the evidence is purely genetic and the value obtained is zero, it cannot be decided whether the gene is situated at the kinetochore or whether it is located on the left or right arm in its close vicinity. Further, this method of calculating the distance of a gene from the kinetochore now needs to be subjected to revision since it is based on the assumption that the kinetochore is the region of the chromosome which holds together the sister chromatids of each chromosome until the end of metaphase II of meiosis (CATCHESIDE, 1951). Now that we know that this is not the case in a large number of organisms it becomes doubtful whether it is in *Neurospora.* A detailed cytological analysis of both divisions of meiosis has recently been made in *N. crassa* (SINGLETON, 1953). SINGLETON's figure of prometaphase II strongly suggests that the most proximal regions of the arms are here also responsible for holding together the sister chromatids (SINGLETON, 1953, Fig. 48). If this is the case, the percentage of recombination does not measure the distance of a gene from the kinetochore but from the distal end of the most proximal region of the arm, which makes the chromosome segment difficult of access to genetic analysis much longer than was supposed before and consequently makes still more remote the possibility of an exact location of genes at the kinetochore by means of this method.

It thus becomes clear that, in *Drosophila*, *Zea* and *Neurospora* genes have not been located at the kinetochore for the simple reason that the genetic methods so far employed have not favoured or permitted their detection.

IV. INTERRELATION AND INTERACTION OF THE KINETOCHORE WITH OTHER CHROMOSOME REGIONS

On the one hand the kinetochore appears now as a region of the chromosome body with a structure, a chemical composition, a type of variation, and a genetic behaviour which do not differ essentially from those of the arms. On the other hand it remains the most well defined and specialized region of the chromosome body dividing it usually into two arms and having a function which is seldom displayed by other chromosome segments. The structural pattern of the arms bears a relation to the position of the kinetochore, and the distribution of most properties exhibited by the arm regions is also conditioned by its location. This dualism is not conflicting. On the contrary, the essential similarity of the kinetochore with the arms is a prerequisite for its interaction with the other regions and at the same time for ensuring a differentiation that results in its partial autonomy.

1. The structural evidence. The gradients

A detailed analysis including the study of the number, size and distribution of the chromomeres of the seven chromosomes of the normal complement of *Secale cereale* at pachytene, revealed the existence of a chromomere size gradient in these chromosomes. Besides this gradient two more, one in the stainability and another in the thickness of the fibrillae, may be observed in these chromosomes (LIMA-DE-FARIA, 1952 a and b).

In *Agapanthus umbellatus* three similar gradients are present at pachytene. At middle prophase II two gradients are also present: one in chromomere size, another in stainability of the fibrillae. At prophase of mitosis there is also a gradient in the stainability of the fibrillae (LIMA-DE-FARIA, 1954).

In the two species the chromomeres are large on both sides of the kinetochore and decrease in size as they approach the ends, where knob formations may be present or not. The fibrillae are also deeply stained and thick on both sides of the kinetochore and their stainability and thickness decrease towards the ends following a parallel course (Figs. 9, 46—48). Recently HYDE (1953) has described a chromomere size gradient at prophase of mitosis in five species of *Plantago* (*P. coronopus*, *P. indica*, *P. psyllium*, *P. lanceolata*, *P. rugelii*) which he considers to be quite similar to that found in rye.

Similar gradients are present at pachytene in other plant species such as *Kniphofia aloides* (BELLING, 1928 b, DARLINGTON, 1933), *Fritillaria Eggeri, F. imperialis, F. verticillata, F. obliqua, F. pontica, F. oranensis* (DARLINGTON, 1935), *Veltheimia viridifolia* (COLEMAN, 1940), *Zea mays* (*cf.* RHOADES, 1940, 1950), *Hordeum vulgare* (MOH and NILAN, 1954), *Sorghum vulgare* and *S. halepense* (HADLEY, 1953), *Plantago ovata* (HYDE, 1953), *Solanum lycopersicum* (BROWN, 1949, BARTON, 1950, GOTTSCHALK, 1951 a), *Notonia grandiflora* (GANESAN, 1939), *Gazania rigens* and *Passiflora* sp. (my own observations). These authors clearly depicted the gradients but they did not mention the phenomenon or just referred to it as a type of heterochromatic differentiation. This phenomenon is thus present in the Liliaceae, Gramineae, Plantaginaceae, Solanaceae, Compositae, Passifloraceae, representing both Monocotyledons and Dicotyledons. It is undoubtedly a widespread phenomenon without necessarily being universal. In certain species of *Lilium* and *Sorghum*, as well as in animals, the gradients are not easily observed. This does not exclude, however, the possibility that the phenomenon responsible for the formation of the gradients is present in these organisms. TJIO and LEVAN (1954) recently found indications that a gradient may also be present in the chromosomes of the mouse.

As pointed out earlier (LIMA-DE-FARIA, 1954), the gradient is a strictly morphological concept. The term *gradient* refers to the gradual decrease in chromomere size, stainability and thickness of the fibrillae observed at prophase of the first and second divisions of meiosis and at the prophase of mitosis. It refers to a system of order *observed* within the chromosome body.

2. The variation observed within the gradients. The chromosome field

Particularly significant for our knowledge of the organization of the chromosome is the fact that the gradients exhibit a form of variation which bears definite relations to specific regions of the chromosome body. The structural evidence on this point has been described in a previous paper (LIMA-DE-FARIA, 1954). Here only a condensed reference is made.

Three sources of evidence reveal that the formation of the gradients depends primarily on the properties of the kinetochore rather than on the properties of the chromosome arms (LIMA-DE-FARIA, 1952 b and 1954).

(a) The formation of the gradients is independent of the particular genetic constitution of the arms of the chromosomes that carry them. The gradients are ex-

hibited in rye as well as in *Agapanthus*, *Zea*, *Solanum*, *Plantago*, etc., by *every chromosome* of the normal complement of the species and for this reason cannot easily be considered to depend for their formation on the particular genetic constitution of the arms of each chromosome. This does not mean that the genetic constitution of each arm of these species may not favour the appearance or expression of the gradients.

(b) The gradients are not only independent of the particular genetic constitution of the arms of the chromosome carrying them, but are also a phenomenon independent of the particular genetic constitution of the species, because similar gradients are found in the chromosomes of such unrelated species as *Secale cereale*, *Agapanthus umbellatus*, *Solanum lycopersicum*, several species of *Plantago*, *Gazania rigens* and others. This, again, does not mean that the genetic constitution of these species may not favour the appearance or expression of the gradients.

(c) Instead, there is a feature common to the chromosomes of the different species. All the gradients observed originate *on both sides of the kinetochore* in the chromosomes of twenty four species listed above and at the three different stages: pachytene, prophase II and prophase of mitosis.

The kinetochore appears to be primarily responsible for the formation of the gradients, yet the decrease, in chromomere size, thickness and stainability of the fibrillae which takes place in the arms, is not solely dependent on the presence of the kinetochore. The chromosome ends appear to be the regions primarily responsible for the variation of the shape of the gradients. Four sources of evidence reveal that the rate of decrease in the properties of the various constituents of the gradients bears a definite relation to the position of the ends (LIMA-DE-FARIA, 1954).

(a) The observed gradients finish at the arm ends and not in the middle of the arms or elsewhere, irrespective of arm length, in twenty-eight of the thirty arms of *Agapanthus umbellatus* at prophase II and in thirteen of the fourteen arms of *Secale cereale* at pachytene.

(b) The lengths of the deeply stained regions, the regions of average stainability and the weakly stained regions (including the knob formations when these are present) bear a definite relation to the distance between the kinetochores and the ends (LIMA-DE-FARIA, 1954, Figs. 4 a, 4 b, 8 a and 8 b).

(c) The two smallest arms in *Agapanthus* having a deviating pattern help us to understand this phenomenon better. When the length of the arm approaches its smallest magnitude, both in this species (LIMA-DE-FARIA, 1954) and in *Fritillaria* (DARLINGTON, 1935) the size of the chromomeres does not decrease so quickly or they just keep a fairly uniform size. This means that the arm length by itself cannot be supposed to be primarily responsible for the variation in shape of the gradients. Ends that are too close to the kinetochore would lack the necessary independence to affect the shape of the gradients to the same extent as when they are situated at longer distances.

(d) It could be supposed that the variation in the shape of the gradients were attributable solely to the variation in the potentiality of the kinetochore from chromosome to chromosome. It can be seen, however, that such a supposition is inconsistent with the fact that *within* the same chromosome, in the case of chromosomes with arms of different lengths, the gradients vary quite differently on both sides of the kinetochore. These results strongly suggest that the distance at which the ends are

found from the kinetochores is the main controlling factor of the variation in shape of the gradients.

Knob formations also apparently play a role in the variation of the gradients, but as, in rye, most of them have a distal position, their role cannot be easily separated from that of the chromosome end in which they are situated. There is, however, in rye a knob formation situated in the middle of the left arm of chromosome V (the nucleolar arm), and knob formations in maize have also sub-median or sub-terminal positions (*cf.* RHOADES, 1950).

The comparison of the left arm of chromosome V of rye with any arm of the same length, as for instance, the left arm of chromosome VI, shows that although these two arms have approximately the same length, the rate of decrease in the properties of the constituents of the gradients is quite different in the left arm of chromosome V from that in the left arm of chromosome VI. An inspection of the gradients of the left arm of chromosome V shows that the existence of the intercalary knob formation and its adjacent weakly stained regions — belonging to the nucleolar organizing region — is the apparent determining factor of the abrupt decrease in the properties of the constituents of the gradients in this arm. Thus, other regions than the ends apparently affect the shape of the gradients.

In rye, another type of specialized regions apparently interferes in a minor degree with the gradients (LIMA-DE-FARIA, 1952 a). These are seriations constituted, in most cases, by a fibrilla pair more weakly stained than the adjacent ones, accompanied on one or both sides by a chromomere pair of larger size than the adjacent ones. These seriations occur at rather regular intervals throughout the whole length of the arm and in all arms. They constitute natural landmarks in the chromosome body which divide the chromosome arms into segments of approximately the same length. They represent regions of the chromosome where the gradients are locally disturbed, without interfering with their general distribution along the arms.

The study of chromomere distribution in *A. umbellatus* revealed that the chromomere pairs dispose themselves along the chromosome body at distances that are of similar magnitude at the different stages, irrespective of the variation in length of the chromosomes from stage to stage (LIMA-DE-FARIA, 1954). This means that the formation of a chromomere appears to interfere with the formation of another chromomere in its close vicinity, since there is, as a rule, the formation of a fibrilla of an almost constant length between two consecutive chromomeres.

Summing up, the structural evidence furnished by the gradients concerning the organization of the chromosome reveals the following main points. (1) The origin of the gradients is related to a definite region of the chromosome — the kinetochore. (2) The rate of decrease in the properties of the constituents of the gradients bears a definite relation to the position of the ends. (3) Not only the ends but also the knob formations and their adjacent regions appear to have an effect on the shape of the gradients. (4) Particular seriations located at regular intervals in the chromosome body represent regions where the gradients are locally disturbed. (5) Moreover, a fairly constant feature of the chromomeres is their spacing along the chromosome.

These results reveal that the *system of order constituted by the chromosome gradients varies in a defined form* under the apparent influence of specialized regions of the chromosome body. Such a behaviour of the gradients constitutes what is called a field effect.

The expression *chromosome field* (LIMA-DE-FARIA, 1954) refers to the fact that the *variation observed within the gradients* takes place in such a way that the *properties exhibited by certain regions in one part of the chromosome bear a definite relation to the properties exhibited by other regions in other parts of the chromosome. These relations are such that the different regions appear to have an unequal share in the formation of the chromosome pattern.* Whereas kinetochores appear to be primarily responsible for the formation of the gradients, chromosome ends, knob formations and their adjacent regions, recurrent seriations and chromomeres or fibrillae apparently interfere with the formation of the gradients on a successively smaller scale. Moreover, — and this is a significant point in the understanding of chromosome organization — the chromosome field *can maintain its pattern when its mass is either reduced or increased.* This is observed: (1) when the arm length varies from chromosome to chromosome or within the same chromosome, and (2) when the arm length of the same chromosome varies from stage to stage. The same pattern is maintained in the short and long arms of rye and *Agapanthus* chromosomes (exceptions are the three arms mentioned above which represent particular cases), and the same essential pattern is also kept in *Agapanthus* chromosomes at pachytene, prophase II, and prophase of mitosis irrespective of the variation of the chromosome length from stage to stage (LIMA-DE-FARIA, 1954). In *Plantago ovata* (HYDE, 1953) the same pattern can also be seen to be maintained at pachytene and at mitotic prophase, irrespective of the variation in length undergone by each chromosome. This is pertinent evidence of well defined interrelations taking place within the chromosome.

3. Non-random distribution of properties along the chromosome body

Before showing that the evidence obtained from the gradients is supported by experimental evidence demonstrating the interaction among chromosome regions, attention should be called to the properties which do not occur at random within the chromosome, since the manifestation of most of them bears a relation to the location of the kinetochore.

Among these properties are:

(1) Quite parallel to the structural gradients is the gradient in crossing

over frequency found after gamma-ray treatment of *Drosophila melanogaster*. In the third chromosome the increase in the crossover values, relative to controls, is greatest on both sides of the kinetochore and becomes progressively lesser towards the ends where it attains negative values (WHITTINGHILL, 1951). Similar gradients were also obtained in *D. melanogaster* after exposure to nitrogen mustard, X-ray treatment and by partial starvation of larvae (WHITTINGHILL, 1948, MULLER, 1925, *cf.* WHITTINGHILL, 1951). Heterologous inversions present in the X and the third chromosome increase crossing over in the second chromosome of *Drosophila*. The crossing over frequency is also highest on both sides of the kinetochore and becomes progressively lesser towards the ends where it again shows a slight increase (SCHULTZ and REDFIELD, 1951). MATHER (1936, 1938, 1939, 1940) had already shown that the frequency of crossing over in both eu- and heterochromatin is dependent on the distance from the kinetochore and that the distribution of chiasmata bears a definite relation to this region.

(2) In *Scilla campanulata*, pseudochiasmata induced by X-rays were found to show non-random distribution along the chromosome. They occurred in a disproportionately low frequency in short arms, their frequency being highest at a greater distance from the kinetochore (REES, 1953).

(3) The same was found to be true for knob formations (LONGLEY, 1939, 1941). At pachytene in maize and teosinte, there is a region in each chromosome arm where the building of knob formations is favoured, the frequency of occurrence of a knob formation depending upon its proximity to this most favourable position. This position is related to the location of the kinetochore.

(4) Non-disjunction at the first pollen mitosis is manifested by certain regions of B chromosomes in plants such as *Secale cereale* (MÜNTZING, 1946, and at the corresponding stage on the female side, HÅKANSSON, 1948), *Anthoxanthum aristatum* (ÖSTERGREN, 1947) and *Festuca pratensis* (BOSEMARK, 1950, 1954). An analysis of their results brings out the following main points. (a) In the B chromosome types of *Secale*, *Anthoxanthum* and *Festuca*, there are two regions keeping the sister chromatids together, one situated in each arm. (b) In each B chromosome type of these three species these two regions are *situated at equal distances from the kinetochore*. (c) These distances are of approximately the same magnitude in the three species. Such regions were not found to be situated just close to the kinetochore or far apart from it. When the arm is small they are situated at the end (short arm of standard frag-

ment in *Secale* and both arms in *Festuca*) and when it is long they are distanced from the kinetochore about $^1/_3$ of the total arm length (long arm of standard fragment of *Secale* and both arms in *Anthoxanthum*). That the manifestation of such a property is under the influence of the kinetochore has already been pointed out by DARLINGTON and THOMAS (1941) and ÖSTERGREN (1947).

(5) Detailed studies following treatment of chromosomes with chemicals and X-rays have revealed that breakage occurs at specific regions of the chromosomes and that the most susceptible zones are located in the vicinity of the kinetochore. In *Vicia faba* (MCLEISH, 1953, 1954, 1955) maleic hydrazide induces breakage in or close to the heterochromatic segments; and with the dose employed 83 per cent of breaks occur in the vicinity of the kinetochore in the nucleolar arm of the largest chromosome. In the same species the proximal regions of the chromosomes also turned out to be favoured by spontaneous breakage (LEVAN and LOTFY, 1950) and the nucleolar organizing region, which is also located not far from the kinetochore, exhibits a very high breakage frequency after treatment with 8-ethoxycaffeine (KIHLMAN and LEVAN, 1951). Breaks also occur more frequently near the kinetochore of *Tradescantia* chromosomes than elsewhere in the arms. This is the case for both spontaneous and X-ray induced breaks (GILES, 1940, SAX and MATHER, 1939). In the X-chromosome of *Drosophila* about one fourth of the breaks observed after X-rays were in the proximal heterochromatic region, which is a non-random distribution according to salivary gland chromosome length (KAUFMANN, 1946). The proximal chromosome regions of several species of *Triticum* are also the most susceptible to X-ray action (CAMARA, WAGNER and GARDÉ, 1950, GARDÉ, 1948, WAGNER, 1950), the ends also showing in this case a high breakage frequency.

Most properties exhibited by the chromosome body, when carefully studied by means of more refined methods, have turned out to bear a relation to the location of the kinetochore.

4. The main objections raised against the concept of chromosome field

Three main objections may be raised against this concept.

It may be argued that in plants like *Antirrhinum* (ERNST, 1939, MECHELKE and STUBBE, 1954) and *Salvia* (LINNERT, 1955) where no gradient is observed, the findings here reported may not apply. It has already been pointed out that the gradients are not of universal occurrence but that they are to be found in a large number of organisms.

Moreover, the gradients have not always the same degree of expression. In *Plantago ovata* and *Solanum lycopersicum* the transition between the large chromomeres and the small ones seems to be more abrupt than it is in rye or *Agapanthus*, and in *Veltheimia* not all chromosomes exhibit the gradients with equal distinctness. The B chromosome types of rye also do not exhibit the gradients (Figs. 49—51). Most biological phenomena display a variation and are not recognized in all organisms, but this does not impair their general validity. For instance, crossing over is not a universal phenomenon either, and the cases where it is absent or reduced, such as in the males of *Drosophila* or in heterochromatic regions do not weaken its acceptance as a general phenomenon. It is by studying first the gradients where they show their best expression and then by successively seeing how far the other patterns diverge from this one that we may obtain an insight into the underlying mechanism. The cases where the gradients are not observed show various degrees of heterochromatic differentiation, such as seriations of large and small chromomeres alternating abruptly. For this reason they may be most useful in disclosing other variants in the degree of interrelation among the various chromosome regions.

A second objection is based on the fact that the numerous rearrangements obtained in maize and *Drosophila* have not revealed, as a rule, any appreciable change in the phenotype of the chromosome sections transferred. If there was an interaction among chromosome regions which was expressed at the chromosome level, it could be supposed that this interaction would show up *immediately* by modifying the phenotype of a chromosome section once it changed position within a chromosome or after transfer to another chromosome. This would only be true if genes did not exist. Such a situation would only be conceivable if we were forced to abandon the gene concept as suggested by GOLDSCHMIDT (1946, 1951, 1954). I have previously emphasized (LIMA-DE-FARIA, 1954) that the concept of chromosome field is not only compatible with the corpuscular theory of the gene but it demands it. The evidence available on the chromosome field only demands that the chromosome is a *functional* unit — and not a *genetic* unit in GOLDSCHMIDT's meaning — where each chromosome segment is a part of the field, interacts with the other regions and is under their control, but where it also keeps its entity. Each segment maintains its genetic constitution but it occupies an equilibrium position that is defined by the interrelations among the various chromosome regions. From the extensive work done with rearrangements, it became clear that *shortly*

after their transfer to another chromosome region, knob formations, heterochromatic and euchromatic segments maintain their identity to a remarkable degree. There are, however, two points which have not, so far, been made equally clear by this work. Once a knob formation or any other segment is transferred to another region of a chromosome two things may happen: either it occupies a new position which is equivalent to the previous one with regard to its relations with the other chromosome regions, or its new position diverges appreciably from the equilibrium position which it formerly occupied. In the first place it is not known whether a chromosome that carries such a newly transferred segment in an equivalent position has bigger chances of survival than one that carries the same segment in a divergent position. For instance, it is unknown whether, in the case of the knob formations of maize which have as a rule a sub-terminal position, chromosomes which after a translocation carried such knob formations at the most proximal regions of the arms would not have bigger difficulties in surviving than chromosomes which got them transferred to the ends or to another region close to the ends. Secondly, another obscure point is the length of time that a segment which changes its position keeps the original pattern. From the study of rearrangements we know that as a rule the original pattern is kept in the first or second generation after the transfer, but it is uncertain whether it is still maintained after an appreciable number of generations *when the new position is not equivalent to the one occupied previously.* It should be remembered that in the classic work of McCLINTOCK (1934), where, in maize, a segment of the nucleolar organizing region was transferred from chromosome 6 to chromosome 9 after an interchange, the segment thus transferred occupied a position which was equivalent to the previous one. According to the figures of McCLINTOCK the distance from the kinetochore was of a similar magnitude, and the distance from the chromosome end was the same, since the newly transferred section carried with it the terminal region. It is thus to be expected that this type of interchange would be more easily recovered than others and that the segment in this new position could continue easily to build a nucleolus.

The third objection raises the question whether the interrelations disclosed by the structural study and the distribution of the chromosome properties have their counterpart in interactions among the various chromosome regions which are *expressed at the chromosome level* and which can be demonstrated experimentally. Experimental evidence of such interactions is now available from many different sources.

5. The experimental evidence

The methods described above for the location of genes suffered from an important limitation: they were mainly conceived and used for the study of the organism phenotype. Progeny tests were used as the routine procedure in gene analysis and the only characters usually described were those exhibited by the body of the organism. Genes manifesting their properties at the chromosome level were for this reason systematically overlooked.

In several organisms a series of experiments can now be seen to reveal the presence of a category of well defined chromosome segments which manifest their properties at the chromosome level and which, as far as the analysis goes, are indistinguishable from the chromosome segments which manifest their properties at the organism level.

Interaction of the kinetochore with other chromosome regions. — Supporting experimental evidence of such an interaction is furnished by the work of ROMAN (1947). In maize, the B chromosome types undergo non-disjunction at the second pollen mitosis. Eight interchanges between A and B chromosomes were obtained after irradiation of pollen. An interchange involving the B chromosome and chromosome 4 resulted in the formation of a chromosome (B^4) possessing the kinetochore and the proximal third of the B type chromosome. This chromosome undergoes non-disjunction at the second pollen mitosis whereas the interchange chromosome bearing the kinetochore of chromosome 4 disjoins normally. Studies of five other A—B interchanges have shown that it is the interchange chromosome which bears the B kinetochore that exhibits non-disjunction in each case.

Additional experimental evidence was obtained in *Sciara.* In the two species *S. coprophila* and *S. similans* at the second spermatocyte division one of the chromosomes, called the »precocious» chromosome, moves earlier to one pole whereas the other chromosomes orientate in the metaphase plate. The precocious chromosome splits into two daughter halves that do not separate, and both pass to the pole which later originates the spermatid. As in the case of B chromosomes of the Gramineae, non-disjunction is accompanied by a selective poleward orientation, and the region that keeps the sister chromatids together is, in *S. coprophila* and *S. similans*, localized somewhere in the arms (METZ, MOSES and HOPPE, 1926, and METZ, 1938). CROUSE (1943) demonstrated by means of induced translocations between the precocious chromosome and the other chromosomes that, in *Sciara coprophila*, the presence of the kine-

tochore and of a short proximal region of the arm of the original precocious chromosome were necessary for the manifestation of the nondisjunction phenomenon. For each male-transmissible translocation involving the precocious chromosome, it has been shown that only one of the translocation chromosomes showed precocity at the second spermatocyte division. In three instances — two in *S. coprophila* and one in *S. reynoldsi* — it has been demonstrated cytologically that the chromosome behaving precociously was the one with the kinetochore of the original precocious chromosome.

In maize, besides the active mobility on the spindle manifested by the kinetochore, other regions localized at the distal region of the arms may show secondary active mobility at the two meiotic divisions. These regions, which are usually found in arms possessing knob formations, are connected with the spindle and move polewards still more rapidly than the kinetochores (RHOADES and VILKOMERSON, 1942, RHOADES, 1952). Plants with chromosomes manifesting secondary active mobility and heterozygous for a long paracentric inversion in chromosome 4 were obtained by RHOADES (1952). This inversion is located in the long arm of chromosome 4, and the proximal break is distant from the kinetochore $^1/_3$ of the total arm length (MORGAN, 1950). Both the normal and inverted chromosome 4 carried a large knob formation in the distal region of the long arm which is included in the inverted segment. Single crossovers within the inversion give rise to two non-crossover monocentric chromatids, one dicentric chromatid which forms a bridge at anaphase I, and an acentric fragment. The knobbed acentric fragment lies passively on the spindle with no indication of active mobility. Active mobility on the spindle is manifested at the same chromatin segments comprising the acentric fragment when they constitute a portion of a whole chromosome 4. It follows that the chromosome region including the kinetochore plays a decisive role in the manifestation of active mobility at the distal arm region where the knob formation is situated. RHOADES (1952) concluded that he had demonstrated that the kinetochore is involved in the manifestation of active mobility at the chromosome ends. This conclusion is most likely but it is not fully valid since, with the rearrangement used, he did not achieve complete isolation of the kinetochore. He has, however, demonstrated beyond doubt, an interaction between two distinct chromosome regions which is expressed at the chromosome level, and in which the kinetochore seems to play the main role.

Interaction of distal regions with other chromosome regions. — Ex-

perimental demonstration of the interaction of natural ends with other chromosome regions was obtained already in the 1930's but its significance in chromosome organization was not realized since it remained for a long time an isolated case. MULLER drew from the beginning attention to this phenomenon which he has subsequently tried to establish with great accuracy. In the study of induced translocations, it was soon found out that smaller chromosomes — like chromosome IV in *Drosophila* — served less often than larger ones for the attachment of translocated pieces of other chromosomes. Most translocations were found to involve mutual breaks in non-homologous chromosomes, the fragments becoming united together at their mutual points of breakage, rather than at any of their original free ends (MULLER, 1932). Subsequently it turned out that terminal deficiencies which should be by far the commonest type of chromosome aberrations, are in reality very rare. Broken ends normally undergo reunion with other broken ends, and if not, the chromosomes carrying them are lost, as a rule, in subsequent generations. In other words, chromosomes that are devoid of natural ends (or telomeres) do not survive in most cases. Thus, the end genes constitute permanent structures that are essential for the normal behaviour of the chromosome (MULLER, 1940, MULLER and HERSKOWITZ, 1954). These results clearly demonstrate an interaction between natural ends and the rest of the chromosome body. The validity of this conclusion has been abundantly illustrated in the studies of chromosomal aberrations induced by X-ray treatment in both *Drosophila* and maize, and studies in other animals and plants suggest that it applies for the chromosomes of most organisms (*cf.* WHITE, 1954). The cases of healing of broken ends found in maize (MCCLINTOCK, 1939, 1941, STADLER, 1939, 1941) and in rye (MÜNTZING and LIMA-DE-FARIA, 1952) indicate that other regions of the chromosome body may take up the function of telomeres, just as, in other cases, chromosome regions other than kinetochores may take up the function of active mobility on the spindle. In maize, the healing process and the secondary active mobility have in common the need of a particular milieu for their manifestation since they are only observable in certain tissues.

In rye it has been precisely shown that the distal regions are necessary for the manifestation of non-disjunction at regions situated far away from them. Similarly, in maize, it has been demonstrated that the distal regions are necessary for the manifestation of secondary active mobility at regions also located far apart. In rye a chromosome derived from the B chromosome type called the standard fragment lacked both a seg-

ment and the property of non-disjunction at the first pollen mitosis (MÜNTZING, 1948 a). A pachytene analysis of this deficient chromosome revealed that it possessed the two arm regions where the manifestation of non-disjunction is located but that it lacked the distal portion of the long arm of the standard fragment (MÜNTZING and LIMA-DE-FARIA, 1952). This finding demonstrates that there is an interaction between the distal portion and the regions where non-disjunction is manifested. The distal heterochromatic segment of the B chromosome in maize is also required for the manifestation of non-disjunction in the B chromosome. The evidence was obtained in strains in which the distal heterochromatic segment was removed by translocation to A chromosomes (ROMAN, 1950, and personal communication).

In a number of races of maize from Latin America and the U. S. A. chromosome 10 has been found to differ from its normal homologue present in other races, by the chromomere structure of the distal region of the long arm and by having attached to the end of this arm an extra segment of chromatin. Active mobility on the spindle at the distal regions of the chromosomes was only manifested in plants homozygous or heterozygous for this particular type of chromosome 10. Sister plants homozygous for the normal 10 did not exhibit secondary active mobility on the spindle. This manifestation of secondary active mobility was not confined to chromosome 10 but was displayed by as many as seven chromosomes of the complement. Moreover, this secondary mobility is much more striking in homozygous than in heterozygous plants for the particular type of chromosome 10 (RHOADES and VILKOMERSON, 1942, and RHOADES, 1952). Their results demonstrate an interaction between the distal region of the particular type of chromosome 10 and the distal regions of the other chromosomes. As this interaction is manifested at the chromosome level, and not at the level of the organism phenotype, it should be noted that the action of this type of chromosome 10 is exercised as far as across the cytoplasm or the nuclear sap, since it reaches regions situated in non-homologous chromosomes.

Interaction among other types of chromosome regions. — In *Drosophila* SCHULTZ (1941) and KAUFMANN (1948) found that in different types of rearrangements, the nucleic acid content of a disc and the appearance of its chromomeres will depend not only on the general environment provided within the nucleus but also on its position within the chromosome. SCHULTZ and CASPERSSON (CASPERSSON, 1950) worked with a stock of *Drosophila* where in the X-chromosome pair one chromosome partner was normal while the other was involved in a trans-

location with the fourth chromosome so that the latter was broken in a heterochromatic region. They found an increase in the nucleic acid content of several bands (situated in the euchromatin), which were brought next to the heterochromatin in the rearrangement chromosome, as compared to the amount found in the same bands of the normal chromosome. A critical review of similar cases is to be found in SERRA (1949).

The most thoroughly studied case of interaction between two distinct chromosome regions — which is manifested at the chromosome level — is the so called Dissociation-Activator (*Ds-Ac*) system investigated in maize by MCCLINTOCK (1949, 1950, 1951 a, 1951 b, 1952, 1953). A chromosome section called *Ds* has its standard location in the short arm of chromosome 9. Two kinds of events occur at this chromosome section which permit us to recognize it. (1) The chromosome breaks at the section into two free segments: one short acentric segment and one long centric segment. (2) Following breakage at this position, fusion of broken ends of sister chromatids may produce a dicentric chromatid and an acentric fragment, or this particular section may simply drop out, thus producing a normal chromosome deficient for the section. The occurrence of the breakage, the formation of the acentric segment and of the centric segment have been observed and described at pachytene (MCCLINTOCK, 1951 b, Figs. 4—8). By the use of one chromosome 9 that carries *Ds* at its standard location and four dominant markers located distally to *Ds* and of an homologous chromosome carrying the recessive alleles and no *Ds*, it is possible to obtain evidence of the behaviour of *Ds* at the level of the organism phenotype, since these dominant markers manifest their action at the organism level. The events at *Ds* that form a dicentric chromatid and an acentric fragment will lead to the elimination of the acentric fragment during a mitotic division. This fragment will form a pycnotic body in the cytoplasm, which subsequently disappears. All the dominant markers carried in the acentric fragment will be removed from the nuclei after such an event has occurred. The sector of tissue derived from these cells will exhibit the collective phenotype of the recessive alleles carried by the homologous chromosome 9 having no *Ds*, and consequently the kernels will be distinctly variegated since some of these dominant markers are concerned with the formation of pigment in the kernels. Thus, by means of cytological and genetic evidence, the two types of events that occur at *Ds*: breakage, and the formation of a dicentric chromatid and an acentric fragment that is subsequently eliminated, have been recognized. *Ds* has its standard

location in the short arm of chromosome 9 but it may be transposed to other regions or to other chromosomes of the complement. In each case the same two kinds of events occur at *Ds*. There is, however, another chromosome section *Ac* (Activator) that must be present in the nucleus if any breakage events are to occur at *Ds*. If it is absent from the nucleus, no detectable events whatsoever occur at *Ds*, wherever it may be located; and no new positions of *Ds* activity appear. *Ac* may occupy different positions within the same chromosome and may be located in different chromosomes. *Ds* is a chromosome section which has an expression both at the chromosome and at the organism level: it exhibits breakage when it interacts with *Ac*, and it interferes with the expression of genes connected with the formation of aleurone and plant colour and the appearance of the endosperm, when transposed next to these genes. This last point does not concern us here as these genes have their expression at the level of the organism phenotype. What is of significance in this connection is that McCLINTOCK has been able to show that a particular chromosome section (*Ac*) is necessary for the manifestation of a property at another definite chromosome section (*Ds*) — this property consisting of a precise series of breakage and reunion events. The fact that *Ac* does not need to be located in the same chromosome as *Ds* demonstrates that the interaction takes place among different chromosomes as in the case of secondary active mobility on the spindle referred to above. *Ds* behaves in all ways like a common gene except that its main activity is expressed at the chromosome level: (1) its position can be defined by linkage studies, (2) it is a well defined chromosome section which can be transposed from one position to another in the chromosome complement by means of structural rearrangements, and (3) it also has an effect on the organism phenotype. When not associated with convenient dominant markers it escapes detection by common progeny tests and that is why its behaviour has been considered unusual, accustomed as geneticists are to detect genes by the analysis of body characters.

BRINK and co-workers (BRINK and NILAN, 1952, BARCLAY and BRINK, 1954, BRINK, 1954) studied in maize another chromosome section, termed »Modulator» (*Mp*) which has its standard position adjacent to the locus of the *P* gene located on the short arm of chromosome 1. Modulator has also an action which is expressed at both the chromosome and the organism level. On one hand, it suppresses the pigment-producing capacity of one of the *P* alleles which is concerned with the pigmentation of pericarp and cob in maize. The mutation of one of the alleles to the

other depends on its absence or presence in the chromosome complement, and its location may be close to or far away from the locus. On the other hand, it interacts with *Ds*, having a similar action to *Ac*, for it promotes the same kind of breakage events at *Ds*. This was recognized by introducing *Ds* and not *Ac* in a stock with Modulator. The results obtained by BRINK and co-workers reveal an interaction, manifested at the chromosome level, between two well defined chromosome sections, one located in the short arm of chromosome 1 (*Mp*) and the other in the short arm of chromosome 9 (*Ds*). While having such an interaction effect at this level Modulator at the same time behaves like a common gene: (1) its position can be defined by crossing over studies, Modulator being in one case situated 25 crossover units from the *P* locus, (2) it can be transposed from one chromosome region to another, and (3) due to the type of interaction with the *P* locus, it belongs to the category of genes known as modifiers (BRINK, 1954).

One of the properties manifested at the chromosome level, that has been studied since the early days of *Drosophila* genetics, is crossing over. It is, thus, not surprising that one of the first examples of the interaction among chromosomes — manifested at the chromosome level — should be found in the effect of inversions on crossing over frequency, although its significance in this connection has not been fully realised. Whatever its mechanism may be, crossing over is, in most organisms, a property manifested by definite chromosome regions expressed by a specific rearrangement of chromosome segments at these regions. MORGAN, BRIDGES and SCHULTZ (1932) established the existence of an interchromosomal effect in *Drosophila melanogaster*. They tested the effect of inverted sections in one chromosome on crossing over in another non-homologous chromosome. Crossing over in chromosome III was studied: (1) when chromosome X and II were normal; (2) when X contained the inversion ClB and chromosome II was normal; (3) when X was normal and chromosome II contained the inversions associated with *Curly*; and (4) when both the X and II contained these inverted chromosome sections. In the presence of inversions in the other chromosomes, crossing over in chromosome III is increased, the increase being greatest in the vicinity of the kinetochore (*cf.* SCHULTZ and REDFIELD, 1951). When both inversions are present simultaneously, the crossing over is highest, the effects of the individual inversions being cumulative.

This result has been subsequently confirmed and enlarged in four species of *Drosophila*. In *D. melanogaster* STEINBERG (1936) studied the effect of autosomal heterozygous inversions on crossing over in the X chromosome. Four different

crosses were made: (1) controls, no inversions in the autosomes; (2) Curly inversions in the second chromosome, third chromosome normal; (3) Payne inversions in the third chromosome, no inversion in the second chromosome; (4) Curly inversions in the second chromosome and Payne inversions in the third chromosome simultaneously. In all four crosses crossing over was measured in the X chromosome by using 7 genes. Autosomal inversions increase crossing over in the X chromosome. The Curly inversions cause an increase in crossing over in the X chromosome that ranges from approximately 10 per cent in the middle of the chromosome to 171,1 per cent at the left end. The Payne inversions cause a uniform increase of approximately 33 per cent throughout the portion of the X chromosome studied. The Curly and Payne inversions when combined affect crossing over in the X chromosome in somewhat the same manner as do the Curly inversions alone. The increase is greatest in the left end where it attains 285,2 per cent. The effect of the combined inversions is greater in every region than is the effect of the inversions taken singly. In the same species GLASS (1933) found that an inversion in the second chromosome increased crossing over in the third chromosome, and STEINBERG and FRASER (1944) studied the effect of the X chromosome inversions on crossing over in the third chromosome. Crossing over was measured in the third chromosome by means of the »rucuca» complex of recessive markers, and the effects of twelve different X chromosome inversions were tested. Eight of the inversions caused an increase in crossing over in the third chromosome, the greatest increase occurred in the vicinity of the kinetochore and fell off sharply on either side. Not all inversions had an effect of the same magnitude. The inversions scute^{8} and yellow^{4}, particularly the latter, show enormous increases; scute^{4} shows little change over the control. ClB and bobbed deficiency, the breaks of whose inversions are very close to each other, show strikingly different patterns of crossing over distribution. SCHULTZ and REDFIELD (1951) compared the effects of the inversions scute^{8} and scute^{4} located in the X chromosome on crossing over in chromosome III. This comparative study gave evidence that heterochromatic regions are important in interchromosomal effects.

In *Drosophila virilis* KOMAI and TAKAKU (1942) studied the effect of two independent inversions, located in the X chromosome, on the crossing over frequencies of other chromosomes. The two inversions when existing together in the heterozygous state increased crossing over in the terminal regions of the third and fifth chromosomes. The amount of this increase varied according to the chromosome, and also according to the region of the chromosome influenced. The influence of naturally-occurring inversions on crossing over in *Drosophila robusta* has been investigated by CARSON (1953) for a series of selected structural heterozygotes. A heterozygous inversion in each arm of the X chromosome greatly increases crossing over in chromosome II, and this effect is intensified by the presence of a heterozygous inversion in the right arm of chromosome III. Crossing over in the X chromosome of *D. pseudoobscura* is modified by different III chromosome homozygous and heterozygous inversion combinations (LEVINE and LEVINE, 1954). It was found that certain, but not all, combinations showed increased crossing over. The amount of crossing over in the X chromosome was found to depend upon the particular III chromosome inversion combination.

As can be seen from the extent of the experiments made, this is one of the best established cases of interaction among regions situated in

different chromosomes that is manifested at the chromosome level. The inversions: (1) may be located in the X, or in any of the autosomes studied, (2) their effect is manifested in the X or in any of the autosomes investigated, (3) both X-ray induced and naturally-occurring inversions exhibit the effect, (4) this type of interaction is found in four different species, and (5) the increase in crossing over values may reach as much as 285,2 per cent.

Following in line with the effect of inversions on crossing over — where the genes situated within the inverted region have their positions changed — is the finding that a definite group of Mendelian genes increases crossing over in non-homologous chromosomes as inversions do. KAPLAN (1953) studied in *Drosophila melanogaster* the effect of a series of Minute genes on somatic crossing over in non-homologous chromosomes. One Minute located on the left arm and two Minutes located on the right arm of the second chromosome increased the percentage of crossing over in the X chromosome. Ml^2 showed the least effect whereas $M(2)S^7$ showed the strongest effect. M(1)o, another Minute, located in the X chromosome, significantly increased the frequency of somatic crossing over of the second chromosome. These Minutes, like heterozygous inversions, increase the frequency of crossing over, especially in the vicinity of the kinetochore (STERN, 1936, KAPLAN, 1953). The induction of crossing over in somatic cells, by sex linked Minutes, by those located on the third chromosome — which were found by STERN (1936) to increase crossing over within this chromosome — and by the three Minutes selected, with no previous bias, from the store of second chromosome Minutes, shows this phenomenon to be a general characteristic of the class of mutants to which they belong (KAPLAN, 1953). These results clearly illustrate the action of a definite class of Mendelian genes on a property manifested at the chromosome level.

The remarkable example of a gene which controls the condensation process of the chromosomes of a species is to be found in the work of LESLEY and FROST (1927). One variety of *Matthiola incana* has chromosomes which are particularly long at the first metaphase of meiosis. Four other varieties have much shorter first metaphase chromosomes. The contrast in chromosome shape between the two types of varieties is very striking, and by crossing them, an F_1 is obtained where the metaphase figures of all individuals have short chromosomes. Long-chromosomed and short-chromosomed plants appear in the F_2 in a 1 : 3 ratio. The long-chromosome character is due to a single recessive gene, which was denoted by *l*. The existence of a gene which controls chro-

mosome condensation is most significant in view of the variation manifested by the gradients and the relation of this variation to definite regions of the chromosome.

The amount of data available at present is enough to demonstrate the interaction between segments of the chromosome body situated in regions separated far apart, or between segments located in different chromosomes. These segments represent numerous and diversified regions of the chromosome body, which in most cases can be well delimited. The number of cases available and the precision and variety of the experiments by which the interaction has been demonstrated supply abundant proof of its occurrence. The concept of chromosome field receives now the experimental support that the structural analysis demanded, and is thus established on a structural and experimental foundation.

GOLDSCHMIDT's criticism of the corpuscular theory of the gene, had the great value of calling our attention to the problems of chromosome organization, but it contained three weak points: (1) it denied the existence of the gene instead of incorporating it into a higher synthesis, (2) it failed to realize the distinction between gene expression at the chromosome and at the organism level, and (3) it lacked a body of structural and genetic evidence to offer instead of the prevalent concepts. The main body of evidence on which GOLDSCHMIDT built his criticism was drawn from the study of position effects involving genes expressing their effects at the organism phenotype, and, for this reason, it is not surprising that he could not attain a coherent picture of the organization of the chromosome (GOLDSCHMIDT, 1946, 1951, 1954).

V. EVOLUTIONARY AND MORPHOGENETIC IMPLICATIONS OF THE CHROMOSOME FIELD

Attention should be called to the evolutionary implications of the chromosome field. As stressed by MATHER and HARRISON (1949) and DARLINGTON and MATHER (1949), selection, whether natural or artificial, distinguishes in its action only between phenotypes. Genetic differences whose effects are expressed in the organism phenotype will be subjected to selection, but genetic differences which are latent, *i. e.*, that are not expressed in the organism phenotype, will be protected from selection. Selection operates on the genetic variability only as it is released in the form of phenotype expression and discriminates between genotypes only to the extent that they give different phenotypes. Thus, at present,

selection is understood as dealing mostly or solely with the organism phenotype. The evidence demonstrating the interaction between the various chromosome regions manifested at the level of the chromosome phenotype, has as an immediate implication the assumption that selection should take place not only at the organism level but also at the chromosome level. Not all kinds of rearrangements or mutations would fit equally well into the system of order that is the chromosome field; and in this way new rearrangements or new mutations would be favoured or not by means of the chromosomes carrying them being preserved or eliminated in subsequent generations, according to whether their location, genetic behaviour, size (in the case of rearrangements), and number, agree with or disturb the established relations within the chromosome field. Thus, before any new rearrangement or mutation is tried at the organism level, it appears that it undergoes a previous trial at the chromosome level. This would mean that the constitution and organization pattern of a chromosome are the prime determinants of its evolutionary trend and that *the genotype of an organism evolves chiefly under conditions defined by the constitution and organization of its chromosomal components.*

A concrete example of such a chromosomal evolutionary trend is to be found in the work carried out in the genus *Drosophila.* In examining the instances where cytological polymorphism has been studied in *Drosophila,* it becomes evident that the cases investigated thus far will fall into one of three categories (WARD, 1952): (1) species in which the gene sequence is extremely stable showing few or no inversions, as *D. virilis* where no inversions were found in over 3000 chromosomes examined by WARTERS (1944); (2) species where the polymorphism is about equally distributed among the various chromosomes of the complement, as *algonquin* (MILLER, 1939), *robusta* (CARSON and STALKER, 1947) and *willistoni* (DA CUNHA, BURLA and DOBZHANSKY, 1950); and (3) species in which the gene sequences are relatively stable with the exception of one chromosome in which there is a great deal of structural variation in the form of inversions. This last category is most significant, for it represents the best known cases of a chromosomal evolutionary trend where the constitution and organization of the chromosome appear as the prime determining factors.

One of the first examples of this type of chromosomal evolution is to be found in *Drosophila pseudoobscura.* Strains of this species coming from the same or from different geographical localities are frequently dissimilar with respect to the gene arrangement in their chromosomes.

The variations in the gene arrangement are due to inversion of chromosome sections. In *pseudoobscura*, the third chromosome was found by DOBZHANSKY and STURTEVANT (1938) to be the most variable one, seventeen different gene arrangements having been recorded; the second chromosome follows next with six; next are the X chromosome with five and the fourth chromosome with two arrangements. The X chromosome is the longest and the third is the next shortest of these four chromosomes (TAN, 1935).

DOBZHANSKY and EPLING (1944) found subsequently that the third chromosome of *Drosophila pseudoobscura* and *D. persimilis* exhibits a much larger number of natural gene arrangements than the other chromosomes of these species and, moreover, that breaks observed in these gene arrangements were much more common in the two short sections 76 and 77 than in the long region comprising sections 63 through 68, which constitutes more than one third of the chromosome. *D. persimilis* was originally treated as race B of *pseudoobscura* and its chromosomes are very similar to those of *pseudoobscura*, only the Y differing in shape (*cf.* TAN, 1935 and PATTERSON and STONE, 1952).

The gene arrangement in the chromosomes of eight strains of *D. nebulosa* has been examined by PAVAN (1946). The species has three pairs of chromosomes: a V-shaped X chromosome, a V-shaped and a rod-like autosome. The gene arrangement in the V-shaped autosome has been found to be constant, and that in the V-shaped X chromosome is also constant, except for a single inversion found in some individuals. On the other hand the gene arrangement in the rod-like chromosome (the third chromosome) is very variable. Seventeen gene arrangements have been identified in this chromosome. The third chromosome is the shortest of the complement.

The mitotic metaphase plate of *Drosophila guaramunu* contains five pairs of rods and a pair of dots. One of the pairs of rods is longer than the other four; this represents the X chromosome. The euchromatic regions of the X and the other four rod autosomes (chromosomes II, III, IV and V) all have approximately the same length in the salivary glands. Despite the similarity in length 13 of the 19 inversions reported were found in chromosome IV and none in the X chromosome, chromosomes II, III, and V containing one, two and three inversions respectively (BRNCIC, 1953).

A total of twenty-two inversions was found in the salivary gland chromosomes of *Drosophila melanica*, three in the right arm of the X chromosome, two in chromosome IV, one in the left arm of the X chromosome, none in chromosome III, and sixteen in chromosome II. This chromosome has developed and maintained a variability not to be found in the others, since the combination and recombination of these 16 inversions resulted in twenty gene sequences in addition to the standard sequence. The arms of the X and chromosomes II, III and IV all have approximately the same length (WARD, 1952).

In the giant strains of *Drosophila montana* (MOORHEAD, 1954) twenty-one independent inversions were found in the salivary gland chromosomes, thirteen of which

occur in chromosome IV. Four other chromosomes of the set, the X, chromosomes II, III and V, although they have approximately the same length as chromosome IV, were found to contain only three, two, two and one inversions respectively. It is somewhat rare that more than one inversion break occurs at the same place. In chromosome IV three inversions have their proximal break localized at the same point. This break point was in each case rechecked several times and there seemed to be no difference cytologically.

One chromosome (chromosome C) of *Drosophila athabasca* (NOVITSKI, 1946), homologous to the highly variable third chromsome of *D. pseudoobscura*, has a disproportionately large number of different gene arrangements. Eleven different gene sequences resulting from a large number of inversions were found in this chromosome. Chromosomes AD (the X chromosome), B and E exhibited only three, two, and two arrangements respectively. Chromosome C is the shortest of the four (STURTEVANT and DOBZHANSKY, 1936).

The chromosomes of the seven species of *Drosophila* just mentioned have in common the following characteristics. (1) One of the chromosomes of the complement is sharply differentiated from the others by a higher plasticity of organization, since it has developed and maintained a large number of arrangements by means of inversions. (2) Of all the chromosomes that constitute the set of these species it is only one pair which shows this phenomenon. (3) In the seven species this particular chromosome was not found to be any of the sex chromosomes. (4) This particular chromosome was also found to be the shortest or the next shortest of the set (the dot like element excluded). This fact has a double significance; on the one hand, it demonstrates that the larger variability is not correlated with a bigger chromosome length and on the other hand, it indicates that a certain chromosome size, expressed by a favourable distance between the various chromosome regions, contributes to the manifestation of this variability. (5) The distribution of the inversion break points was found to deviate from random in the third chromosome of *D. pseudoobscura*, and to show an obvious concentration at the distal region in the corresponding chromosome of *D. athabasca* (NOVITSKI, 1946). The features common to this phenomenon strongly indicate that the organization pattern of one of the chromosomes of the set is the main factor of its differential variability.

The detail and extent of the analysis of this phenomenon and the similarity that it displays in several different species of *Drosophila* makes it a reliable example of a chromosomal evolutionary trend which cannot be easily explained in terms of interaction between genes taking place only at the level of the organism phenotype and in terms of selection taking place only at a similar level. DOBZHANSKY and EPLING (1944), PAVAN (1946), NOVITSKI (1946), WARD (1952) and PATTERSON

and STONE (1952) have realized for a long time the difficulty of explaining this phenomenon by our common ideas on natural selection, but as they lacked knowledge of the experimental evidence on the interaction among chromosome regions expressed at the chromosome level with the subsequent implication of selection taking place at this level, they could not make these results fit within the framework of the available ideas on evolution.

The formation of easily recognizable patterns is to be found in inorganic matter and organisms. In the study of morphogenesis a great difficulty has been to find a link between both levels of organization, since the egg already shows an organization which cannot easily be explained on purely molecular terms, and since genetics did not furnish a picture of the chromosome where an organized pattern could be found.

EPHRUSSI (1953) has clearly emphasized that cytoplasmic mechanisms or nucleo-cytoplasmic interactions as they are conceived at present may be an instrument of somatic differentiation, but none of them can be considered its primary factor. He was led to seek the primary cause of differentiation in the initial anisotropy of the egg expressed by its polarity and symmetry, but he considered it a reflection of the activity of a more fundamental unit. This unit could not be easily ascertained at that time for as EPHRUSSI states: »so long as the basis of genetics is the study of differences, it cannot be expected to give us an undistorted picture of the cell as a whole. The integrated character of the cell, which is its fundamental property, is bound to escape our notice most of the time». According to WADDINGTON (1951, 1953) there are many examples of patterns arising within single cells and thus demanding a theory dealing in sub-cellular units. He has also stressed that a purely molecular mechanism can hardly account for the formation of pattern. DALCQ (1951) and BONNER (1952) also pointed out the difficulty in explaining the pattern of the egg without a sub-cellular unit which also has an organized pattern. The unit that comes after the cell is the chromosome and the present finding of an organized chromosome pattern, accompanied by interactions among its regions, furnishes the framework by which the molecular level is integrated into an organized system which establishes the morphogenetic link between on the one hand the molecular and on the other hand the cell and organism level.

This work has been supported by a research grant from the Swedish Natural Science Research Council.

SUMMARY

(1) There are at present five main criteria by which the limits of the kinetochore may be determined. These criteria used together allow an accurate delimitation of the kinetochore in most organisms and at nearly all stages.

(2) The fundamental pattern of the kinetochore consists of three zones each with a compound structure. At pachytene each zone has a length that is equivalent to the diameter of the short arms of the mitotic metaphase chromosomes of many plant and animal species. The exterior zone is the least conspicuous, and the median zone the most conspicuous of the three. Each zone was found to be composed of chromomeres, or fibrillae, or of both.

(3) At metaphase of mitosis the kinetochore structure may be seen to be more complex than previously described. In root tip chromosomes of *Hyacinthus orientalis* the interior zone also displays a compound structure. In addition to the fibrilla pairs of the exterior zone and the chromomere pairs of the median zone, one chromomere or chromomere pair is located at the centre of the kinetochore.

(4) The three zones of the kinetochore are present also at metaphase I, and the interior zone has, as well, a compound structure. In *Tradescantia virginiana*, at this stage, in a chromosome of a bivalent the short arm is attached to one half of the kinetochore in upward direction, consisting of a fibrilla (exterior zone), one chromomere (median zone), a fibrilla, one smaller chromomere and a fibrilla (interior zone); the other half of the kinetochore in downward direction, is formed by the same three zones and is attached to the long arm. The same type of kinetochore structure is present at metaphase I in species with chromosomes having median or sub-median kinetochores, such as *Tradescantia bracteata*, and in species having chromosomes with strictly terminal kinetochores such as *Mecostethus grossus*. At metaphase II, the three zones may be seen in the chromosomes of this last species.

(5) The two main difficulties which prevented an accurate study of the kinetochore at metaphase and anaphase I of meiosis have been removed. (a) The existence of the folding of the kinetochore at metaphase I is clearly displayed in tetravalents and bivalents of *Tradescantia virginiana*. At this stage the kinetochore is seen uniting the short and long arm of each chromosome and is sharply folded at the interior zone. (b) At anaphase I, in both *Tradescantia bracteata* and *T. virginiana*, the kinetochore can be delimited with exactitude owing to the parti-

cular behaviour of the most proximal regions of the arms. Due to the finding of a structural pattern which conforms with that found at other stages, the demonstration of the folding, and the presence of a criterion of delimitation, the structure of the kinetochore can now be established with accuracy at metaphase and anaphase I of meiosis.

(6) The homology of the three kinetochore zones may now be accurately established at mitosis and at both divisions of meiosis. Moreover, this fundamental pattern occurs among remotely related groups of plants and animals.

(7) The matrix is involved in a binding process which results in the holding together of the sister chromatids of each chromosome at the most proximal regions of the arms. This phenomenon is observed at prophase and metaphase of mitosis (*Galtonia candicans*, *Vicia faba*), at anaphase I (*Tradescantia virginiana*, *T. bracteata*), prophase II (*Agapanthus umbellatus*), and metaphase II of meiosis (*Fritillaria meleagris*, *Rhoeo discolor*, *Mecostethus grossus*). It can equally be seen in chemically treated chromosomes at metaphase of mitosis (*Allium Cepa*, *Hyacinthus orientalis*). This phenomenon occurs in chromosomes with median, sub-median, sub-terminal and strictly terminal kinetochores and can be recognized in many different organisms. The kinetochore is already divided at metaphase of mitosis and at anaphase I of meiosis.

(8) The fact that crossing over is less frequent in the vicinity of the kinetochore than elsewhere may now have its natural explanation, since the two sister chromatids of each chromosome keep undivided at these regions until metaphase II of meiosis. Both the kinetochores and the most proximal regions of the arms of a bivalent are thus likely to be segregated from one another at division I of meiosis, and not only the kinetochores as previously assumed. The method of determining the distance of a gene from the kinetochore, which in *Neurospora* was based on this assumption, now needs to be revised.

(9) At anaphase I of meiosis in *Tradescantia bracteata*, *T. virginiana*, *Secale cereale* and other species, two distinctly separated kinetochores, instead of one, can be seen to be present in each chromosome. This means that the surface of the kinetochore available for contact with the spindle is greatly increased.

(10) Four main regions with distinct cycles of division are present in the chromosome body. Within a chromosome the median regions, the chromosome ends, the kinetochores and the most proximal regions of the arms were found to divide at different stages.

(11) The kinetochore is negatively heteropycnotic at metaphase of mitosis and at metaphase and anaphase I of meiosis.

(12) That the kinetochore is essentially a tandem reversed repeat may be seen in the chromosomes of: *Secale cereale* at pachytene, *Hyacinthus orientalis* at metaphase of mitosis, and *Tradescantia virginiana* at metaphase I, where this feature is most striking due to the folding of the kinetochore. Experimental evidence showing that both halves of the kinetochore are also functionally alike is available in rye and maize.

(13) Breakage events at the kinetochore cannot be recognized as distinct from breakage in the arms. The kinetochore can exhibit a higher degree of spontaneous breakage than other arm regions and reacts to X-rays and various chemicals in a similar way. The occurrence of breakage at the kinetochore is a necessary condition for its structural variation, and the evidence available suggests that breakage may take place at the three kinetochore zones. Chromosomes whose kinetochores are larger and smaller than those of the other chromosomes of the complement are found in several species. Kinetochores which are functionally dissimilar from those of other chromosomes are also present in various organisms.

(14) The structural appearance of kinetochores bears a relation to chromosome size following apparently two rules. (a) The species with the largest chromosomes show the most clear structure of the kinetochore. (b) Within a chromosome complement the largest chromosomes tend to exhibit the clearest kinetochore structure. In both cases the underlying cause is to be sought in the presence of a larger kinetochore in these chromosomes.

(15) The Feulgen test was applied to the chromosomes of *Tradescantia virginiana* and *T. bracteata* at metaphase and anaphase I of meiosis, two stages where the kinetochore is involved in chromosome movements. Both the fibrillae and the chromomeres of the kinetochore stained intensely by the Feulgen reaction. In *T. virginiana* at metaphase I, the total length of the kinetochore is circa five micra. The large size of the kinetochore and the intensity of the reaction, allied to the previous demonstration of DNA in the kinetochores of pachytene and diplotene chromosomes, is ample confirmation of the existence of DNA in this chromosome region. Such a result is particularly relevant to the genetics of the kinetochore due to the role attributed to nucleic acids in gene structure and gene reproduction.

(16) Genes have not so far been localized at the kinetochore for the simple reason that the genetic methods employed have not favoured or

permitted their detection. The genetic methods of analysis were mostly used in the study of the characters exhibited by the body of the organism, and for this and other reasons (such as the difficulties in delimiting the kinetochore in salivary gland chromosomes) genes that may possibly have been located at the kinetochore were systematically overlooked or could not be localized at this region with any exactitude.

(17) At the same time as the kinetochore is found to have a structure, a division cycle, a type of variation, a chemical composition and a genetic behaviour which do not differ essentially from those of the arms, it also turns out to be the region of the chromosome body to which the pattern and the properties of the arms stand in a definite relation.

(18) Gradients in chromomere size, accompanied in most cases by gradients in the thickness and stainability of the fibrillae — which originate on both sides of the kinetochore — are found in a large number of organisms at both mitosis and the two divisions of meiosis. They constitute a system of order which varies in a defined form under the apparent influence of specialized regions of the chromosome body. The expression chromosome field refers to the fact that the properties exhibited by certain regions in one part of the chromosome bear a definite relation to the properties exhibited by other regions in other parts of the chromosome. These relations are such that the different regions appear to have an unequal share in the formation of the chromosome pattern. Of particular significance in chromosome organization is the finding that the pattern which the gradients form is maintained when the chromosome mass is either reduced or increased. This is observed: (a) when the arm length varies from chromosome to chromosome or within the same chromosome, and (b) when the arm length of the same chromosome varies from stage to stage. The structural analysis of the arms furnishes pertinent evidence of the existence of well defined interrelations within the chromosome. Moreover, most properties exhibited by the chromosome body, when carefully studied by means of more refined methods have turned out to bear a relation to the location of the kinetochore.

(19) The interrelations disclosed by the structural study of the arms and the distribution of the chromosome properties can now be shown to have their counterpart in interactions among the various chromosome regions which are *expressed at the chromosome level.* Experimental evidence of such interactions is provided by many different sources. The amount of data available at present goes to demonstrate the interaction between segments of the chromosome body situated in widely

separated regions or between segments located in different chromosomes. These segments represent numerous and diversified regions of the chromosome body (segments including the kinetochore, telomeres, and other segments of the arms) which in most instances can be well delimited. The number of cases studied and the precision and variety of the experiments by which the interaction has been demonstrated supply abundant proof of its occurrence. The concept of chromosome field is thus established on a structural and experimental foundation.

(20) At present selection is understood to deal mostly or solely with the organism phenotype. The evidence demonstrating the interaction between the various chromosome regions manifested at the level of the chromosome phenotype, has as an immediate implication the assumption that selection should also take place at this level. Examples of a chromosomal evolutionary trend, available in the genus *Drosophila*, which for a long time could hardly be explained in terms of the accepted evolutionary concepts, are most easily interpreted by assuming selection at the chromosome level.

Literature cited

ANDERSON, E. G. and RANDOLPH, L. F. 1945. Location of the centromeres on the linkage maps of maize. — Genetics 30: 518—526.

AUERBACH, C. 1947. Abnormal segregation after chemical treatment of *Drosophila*. — Genetics 32: 3—7.

BAILEY, P. C. 1954. A further study of the chromosome morphology of some species of *Trillium*. — Bull. Torrey Bot. Club 81: 68—75.

BARBER, H. N. 1941. Chromosome behaviour in *Uvularia*. — J. Genetics 42: 223—257.

BARCLAY, P. C. and BRINK, R. A. 1954. The relation between modulator and activator in maize. — Proc. Nat. Acad. Sci. 40: 1118—1126.

BARTON, D. W. 1950. Pachytene morphology of the tomato chromosome complement. — Amer. J. Bot. 37: 639—643.

BAUER, H. 1933. Mikroskopisch-chemischer Nachweis von Glykogen und einigen anderen Polysacchariden. — Zschr. f. mikr.-anat. Forsch. 33: 143—160.

BAUER, H. and BEERMANN, W. 1952. Der Chromosomencyclus der Orthocladiinen (Nematocera, Diptera). — Z. Naturforschung 7 b: 557—563.

BEERMANN, W. 1952. Chromomerenkonstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. — Chromosoma 5: 139—198.

BELLING, J. 1928 a. The ultimate chromomeres of *Lilium* and *Aloë* with regard to the numbers of genes. — Univ. Calif. Publ. Bot. 14: 307—318.

— 1928 b. Contraction of chromosomes during maturation divisions in *Lilium* and other plants. — Univ. Calif. Publ. Bot. 14: 335—343.

BENOIT, J. et KEHL, R. 1939. Les centromères au cours de la spermatogenèse de la souris. — C. R. Soc. de Biol. 131: 329—332.

BERGER, C. A. and WITKUS, E. R. 1943. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea*, with comparative observations on *Allium Cepa*. — Bull. Torrey Bot. Club 70: 457—467.

BONNER, J. T. 1952. Morphogenesis. An essay on development. — Princeton. 296 p.

BOSEMARK, N. O. 1950. Accessory chromosomes in *Festuca pratensis* HUDS. — Hereditas 36: 366—368.

— 1954. On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. — Hereditas 40: 346—376.

BRIDGES, C. B. 1935. Salivary chromosome maps with a key to the banding of the chromosomes of *Drosophila melanogaster*. — J. Heredity 26: 60—64.

BRIDGES, C. B. and BREHME, K. S. 1944. The mutants of *Drosophila melanogaster*. — Carn. Inst. Wash. Publ. 552: 1—257.

BRINK, R. A. 1954. Very light variegated pericarp in maize. — Genetics 39: 724—740.

BRINK, R. A. and NILAN, R. A. 1952. The relation between light variegated and medium variegated pericarp in maize. — Genetics 37: 519—544.

BRNCIC, J. D. 1953. Chromosomal variation in natural populations of *Drosophila guaramunu*. — Z. Ind. Abst.- u. Vererb. 85: 1—11.

BROWN, S. W. 1949. The structure and meiotic behavior of the differentiated chromosomes of tomato. — Genetics 34: 437—461.

BURLA, H. 1950. Die Chromosomensätze der in der Schweiz vorkommenden *Drosophila*-Arten *D. helvetica, D. kuntzei, D. limbata, D. testacea, D. littoralis* und *D. nigrosparsa*. — Arch. Julius Klaus-Stif. 25: 496—504.

CALDECOTT, R. S. 1953. The effects of X-radiation on genic material. — Northwest Science 27: 25—32.

CALLAN, H. G. and MONTALENTI, G. 1947. Chiasma interference in mosquitoes. — J. Genetics 48: 119—134.

CAMARA, A. 1939. Efeitos do calor sobre a microsporogenese do *Secale cereale*. — Scientia Genetica 1: 86—102.

— 1953. Posicao actual do problema do centromero. — Genetica Iberica 5: 67—99.

CAMARA, A., NORONHA WAGNER, M. and GARDÉ, A. 1950. Location of breaks induced by X-rays in chromosomes of *Triticum*. — Genetica Iberica 2: 3—13.

CARPENTIER, S. and PACAULT, A. 1949. Action mitoclasique de quelques carbures cycliques. Relation entre cette action et l'aromaticité des carbures. — Rev. Cytologie Biol. Veg. 11: 305—313.

CARSON, H. L. 1953. The effects of inversions on crossing over in *Drosophila robusta*. — Genetics 38: 168—186.

CARSON, H. L. and STALKER, H. D. 1947. Gene arrangements in natural populations of *Drosophila robusta* STURTEVANT. — Evolution 1: 113—133.

CASPERSSON, T. O. 1950. Cell growth and cell function. A cytochemical study. — New York. 185 p.

CATCHESIDE, D. G. 1951. The genetics of micro-organisms. — London. 223 p.

CHIARUGI, A. 1949. Saggio di una revisione citosistematica della flora italiana. I. Il tetraploidismo della *Bellevalia Webbiana* PARL. e il suo diritto di cittadinanza nella flora italiana. — Caryologia 1: 362—377.

COLEMAN, L. C. 1940. The cytology of *Veltheimia viridifolia*, JACQ. — Amer. J. Bot. 27: 887—895.

COLEMAN, L. C. 1948. The cytology of some western species of *Trimerotropis* (Acrididae). — Genetics 33: 519—528.

CROUSE, H. V. 1943. Translocations in *Sciara*; their bearing on chromosome behavior and sex determination. — Missouri Agric. Exp. Stat. Res. Bull. 379: 1—75.

DA CUNHA, A. B., BURLA, H., and DOBZHANSKY, TH. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. — Evolution 4: 212—235.

DALCQ, A. M. 1951. Form and modern embryology. — Aspects of form, edited by L. L. Whyte: 91—120.

D'AMATO, F. 1949. Agglutinazione cromosomica (»stickiness») quale effetto citologico caratteristico di due composti cromici. — Caryologia 2: 101—110.

— 1950. The chromosome breaking activity of chemicals as studied by the *Allium Cepa* test. — Pubbl. Staz. Zool. Napoli, Suppl. 22: 1—16.

DARLINGTON, C. D. 1933. Meiosis in *Agapanthus* and *Kniphofia*. — Cytologia 4: 229—240.

— 1934. Anomalous chromosome pairing in the male *Drosophila pseudo-obscura*. — Genetics 19: 95—118.

— 1935. The internal mechanics of the chromosomes. II. Prophase pairing at meiosis in *Fritillaria*. — Proc. Royal Soc. London, B, 118: 59—73.

— 1936. The analysis of chromosome movements. I. *Podophyllum versipelle*. — Cytologia 7: 242—247.

— 1937. Recent Advances in Cytology. — London. 671 p.

— 1938. Chromosome behaviour and structural hybridity in the Tradescantiae. II. — J. Genetics 35: 259—280.

— 1939. Misdivision and the genetics of the centromere. — J. Genetics 37: 341—364.

— 1940 a. The origin of iso-chromosomes. — J. Genetics 39: 351—361.

— 1940 b. The causal sequence of meiosis. II. Contact points and cossing-over potential in a triploid *Fritillaria*. — J. Genetics 41: 35—48.

— 1947. Nucleic acid and the chromosomes. — Symp. Soc. Exp. Biol. 1: 252—269.

— 1949. The working units of heredity. — Proc. Eighth Int. Cong. Genetics, Hereditas Suppl. vol.: 189—200.

DARLINGTON, C. D. and KOLLER, P. C. 1947. The chemical breakage of chromosomes. — Heredity 1: 187—221.

DARLINGTON, C. D. and LA COUR, L. F. 1950. Hybridity selection in *Campanula*. — Heredity 4: 217—248.

— and — 1953. The classification of radiation effects at meiosis. — Supp. Heredity 6: 41—55.

DARLINGTON, C. D. and MATHER, K. 1949. The elements of genetics. — London. 446 p.

DARLINGTON, C. D. and THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. — Proc. Royal Soc. London, Ser. B 130: 127—150.

DOBZHANSKY, TH. and EPLING, C. 1944. Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. — Carneg. Inst. Washington Publ. 554: 1—183.

DOBZHANSKY, TH. and STURTEVANT, A. H. 1938. Inversions in the chromosomes of *Drosophila pseudoobscura*. — Genetics 23: 28—64.

DOWRICK, G. J. 1952. The chromosomes of *Chrysanthemum*, I: the species. — Heredity 6: 365—375.

ELLERSTRÖM, S. and TJIO, J. H. 1950. Note on the chromosomes of *Phleum echinatum*. — Bot. Not. 1950: 463—465.

EPHRUSSI, B. 1953. Nucleo-cytoplasmic relations in micro-organisms. Their bearing on cell heredity and differentiation. — Oxford. 127 p.

ERNST, H. 1939. Zytogenetische Untersuchungen an *Antirrhinum majus* L. — Zeit. f. Bot. 34: 81—111.

FERNANDES, A. 1937. Sur l'origine du *Narcissus dubius* GOUAN. — Bol. Soc. Brot. 12: 93—118.

FRANKEL, O. H. 1949. A self-propagating structural change in *Triticum*. II. The reproductive cycle. — Heredity 3: 293—317.

GALL, J. G. 1954. Lampbrush chromosomes from oocyte nuclei of the newt. — J. Morph. 94: 283—352.

GANESAN, D. 1939. Cytological studies in a chromosome ringforming diploid *Notonia grandiflora* DC. — J. Genetics 38: 493—516.

GARBER, E. D. 1947. The pachytene chromosomes of *Sorghum intrans*. — J. Heredity 38: 251—252.

GARDÉ, A. 1948. Análise da accao dos raios X sobre os cromosomas do *Triticum monococcum* L. — Agron. Lusitana 10: 25—73.

GARDÉ, A. and MALHEIROS-GARDÉ, N. 1949. Contribuicao para o estudo cariologico da familia Umbelliferae. I. — Agron. Lusitana 11: 91—140.

GEITLER, L. 1933. Das Verhalten der Chromozentren von *Agapanthus* während der Meiose. — Österr. Bot. Z. 82: 277—282.

— 1951. Der Bau des Zellkerns von *Navicula radiosa* und verwandten Arten und die präanaphasische Trennung von Tochtercentromeren. — Österr. Bot. Z. 98: 206—214.

GILES, N. 1940. Spontaneous chromosome aberrations in *Tradescantia*. — Genetics 25: 69—87.

GLASS, H. B. 1933. A study of dominant mosaic eye-colour mutants in *Drosophila melanogaster*. II. Tests involving crossing-over and non-disjunction. — J. Genetics 28: 69—112.

GOLDSCHMIDT, R. B. 1946. Position effect and the theory of the corpuscular gene. — Experientia 2: 1—40.

— 1951. The theory of the gene. Chromosomes and genes. — Cold Spring Harb. Symp. Quant. Biol. 16: 1—11.

— 1954. Different philosophies of genetics. — Proc. 9th Int. Cong. Genetics 1: 83—99.

GOTTSCHALK, W. 1951 a. Untersuchungen am Pachytän normaler und röntgenbestrahlter Pollenmutterzellen von *Solanum lycopersicum*. — Chromosoma 4: 298—341.

— 1951 b. Der Vergleich von röntgen- und chemisch induzierten Chromosomenmutationen im Pachytän von *Solanum lycopersicum*. — Chromosoma 4: 342—358.

HADLEY, H. H. 1953. Cytological relationships between *Sorghum vulgare* and *S. halepense*. — Agr. Journal 45: 139—143.

HAQUE, A. 1953. The irradiation of meiosis in *Tradescantia*. — Heredity Supp. 6: 57—75.

HASKELL, G. and MARKS, G. E. 1952. Chromosome ecology of British *Galinsoga* species. — The New Phytologist 51: 382—387.

HEITZ, E. 1936. Die Nukleal-Quetschmethode. — Ber. der Deutschen Bot. Ges. LIII: 870—878.

HUSKINS, C. L. 1947. The subdivision of the chromosomes and their multiplication in nondividing tissues: possible interpretations in terms of gene structure and gene action. — Amer. Nat. 81: 401—434.

HYDE, B. B. 1953. Differentiated chromosomes in *Plantago ovata*. — Amer. J. Bot. 40: 809—815.

HÅKANSSON, A. 1940. Die Meiosis bei haploiden Pflanzen von *Godetia Whitneyi*. — Hereditas 26: 411—429.

— 1948. Behaviour of accessory rye chromosomes in the embryo-sac. — Hereditas 34: 35—59.

INABA, F. 1941. Polyploidy in *Habrobracon* induced by colchicine treatment. — Cytologia 12: 66—78.

IWATA, J. 1940. Studies on chromosome structure. II. The attachment chromomeres in the meiotic chromosomes. — Japanese J. Bot. 10: 375—382.

KAPLAN, W. D. 1953. The influence of minutes upon somatic crossing over in *Drosophila melanogaster*. — Genetics 38: 630—651.

KARPECHENKO, G. D. 1940. On the transverse division of chromosomes as a result of colchicine treatment. — C. R. A. S. URSS 29: 404—406.

KAUFMANN, B. P. 1946. Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. — J. Exp. Zool. 102: 293—320.

— 1948. Chromosome structure in relation to the chromosome cycle. II. — Bot. Review 14: 57—126.

KIHLMAN, B. and LEVAN, A. 1951. Localized chromosome breakage in *Vicia faba*. — Hereditas 37: 382—388.

KOLLER, P. C. 1947. The experimental modification of nucleic acid systems in the cell. — Symp. Soc. Exp. Biol., Nucleic Acid, 1: 270—290.

KOMAI, T. and TAKAKU, T. 1942. On the effect of the X-chromosome inversions on crossing over in *Drosophila virilis*. — Cytologia 12: 357—365.

KOSLOV, V. E. 1937. Observations on the kinetochore of mitotic chromosomes. — Biol. Zhurnal 6: 759—766.

KURITA, M. 1953. A study of chromosomes in *Nothoscordum fragrans* (VENT.) KUNTH. — Memoirs Ehime Univ. Sect. II, 1: 379—387.

LA COUR, L. F. 1950. Compound constrictions. — Heredity 4: 243—248.

LESLEY, M. M. and FROST, H. B. 1927. Mendelian inheritance of chromosome shape in *Matthiola*. — Genetics 12: 449—460.

LEVAN, A. 1940. Framställning av tetraploid rödklöver. — Sveriges Utsädesförenings Tidskrift 1940: 115—124.

— 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas 28: 177—211.

— 1949. The influence on chromosomes and mitosis of chemicals, as studied by the *Allium* test. — Proc. Eighth Int. Cong. Genetics, Hereditas Suppl. vol.: 325—337.

LEVAN, A. and EMSWELLER, S. L. 1938. Structural hybridity in *Nothoscordum fragrans*. — J. Heredity 29: 291—294.

LEVAN, A. and LOTFY, T. 1950. Spontaneous chromosome fragmentation in seedlings of *Vicia faba*. — Hereditas 36: 470—482.

LEVINE, R. P. and LEVINE, E. P. 1954. The genotypic control of crossing over in *Drosophila pseudoobscura*. — Genetics 39: 677—691.

LEWIS, E. B. 1945. The relation of repeats to position effect in *Drosophila melanogaster*. — Genetics 30: 137—166.

LIMA-DE-FARIA, A. 1949 a. The structure of the centromere of the chromosomes of rye. — Hereditas 35: 77—85.

— 1949 b. Genetics, origin and evolution of kinetochores. — Hereditas 35: 422—444.

LIMA-DE-FARIA, A. 1950. The Feulgen test applied to centromeric chromomeres. — Hereditas 36: 60—74.

— 1952 a. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5: 1—68.

— 1952 b. The chromomere size gradient of the chromosomes of rye. — Hereditas 38: 246—248.

— 1953. The regions of special cycle of division of *Agapanthus* chromosomes. — Chromosoma 6: 33—44.

— 1954. Chromosome gradient and chromosome field in *Agapanthus*. — Chromosoma 6: 330—370.

— 1955 a. The division cycle of the kinetochore. — Hereditas 41: 238—240.

— 1955 b. Structure and behaviour of a chromosome derivative with a deleted kinetochore. — Chromosoma 7: 51—77.

— 1955 c. Structural differentiation of the kinetochore in rye and *Agapanthus*. — Chromosoma 7: 78—89.

— 1955 d. Structure, division and delimitation of the kinetochore in *Tradescantia*. — Hereditas 41: 209—226.

LINNERT, G. 1955. Die Struktur der Pachytänchromosomen in Euchromatin und Heterochromatin und ihre Auswirkung auf die Chiasmabildung bei *Salvia*-Arten. — Chromosoma 7: 90—128.

LONGLEY, A. E. 1939. Knob positions on corn chromosomes. — J. Agr. Res. 59: 475—490.

— 1941. Knob positions on teosinte chromosomes. — J. Agr. Res. 62: 401—413.

MATHER, K. 1932. Chromosome variation in *Crocus*. I. — J. Genetics 26: 129—142.

— 1934. The behaviour of meiotic chromosomes after X-irradiation. — Hereditas 19: 303—322.

— 1936. The determination of position in crossing-over. I. *Drosophila melanogaster*. — J. Genetics 33: 207—235.

— 1938. Crossing-over. — Biol. Rev. 13: 252—292.

— 1939. Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. — Genetics 24: 413—435.

— 1940. The determination of position in crossing-over. III. The evidence of metaphase chiasmata. — J. Genetics 39: 205—223.

MATHER, K. and HARRISON, B. J. 1949. The manifold effect of selection. — Heredity 3: 1—52.

MATSUURA, H. 1941. Chromosome studies on *Trillium kamtschaticum* PALL. XIII. The structure and behavior of the kinetochore. — Cytologia 11: 369—379.

MATTHEY, R. 1949. La formule chromosomique et la méiose chez *Apteromantis bolivari* WERNER. (*Mantidae-Amelinae*). — Arch. Julius Klaus-Stif. 24: 114—123.

MCCLINTOCK, B. 1933. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. — Z. Zellforschung 19: 191—237.

— 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. — Z. Zellforschung u. mikrosk. Anat. 21: 294—328.

— 1938. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. — Genetics 23: 315—376.

— 1939. The behavior in successive nuclear divisions of a chromosome broken at meiosis. — Proc. Nat. Acad. Sci. 25: 405—416.

McCLINTOCK, B. 1941. The stability of broken ends of chromosomes in *Zea mays*. — Genetics 26: 234—282.

— 1949. Mutable loci in maize. — Annual Report, Carneg. Inst. Washing. Year Book No. 48: 142—154.

— 1950. Mutable loci in maize. — Annual Report, Carneg. Inst. Washing. Year Book No. 49: 157—167.

— 1951 a. Mutable loci in maize. — Annual Report, Carneg. Inst. Washing. Year Book No. 50: 174—181.

— 1951 b. Chromosome organization and genic expression. — Cold Spring Harbor Symp. Quant. Biol. 16: 13—47.

— 1952. Mutable loci in maize. — Annual Report, Carneg. Inst. Washing. Year Book No. 51: 212—219.

— 1953. Induction of instability at selected loci in maize. — Genetics 38: 579—599.

McLEISH, J. 1953. The action of maleic hydrazide in *Vicia*. — Heredity Suppl. 6: 125—147.

— 1954. The consequences of localized chromosome breakage. — Heredity 8: 385—407.

— 1955. Radiation sensitivity and the mitotic cycle in *Vicia faba*. — Nature 175: 890.

MECHELKE, F. and STUBBE, H. 1954. Studien an mutablen Genen. I. *Antirrhinum majus* L. Mut. *graminifolia*. — Z. Ind. Abst.- u. Vererb. 86: 224—248.

MELANDER, Y. 1950. Studies on the chromosomes of *Ulophysema öresundense*. — Hereditas 36: 233—255.

METZ, C. W. 1938. Chromosome behavior, inheritance and sex determination in *Sciara*. — Amer. Nat. 72: 485—520.

METZ, C. W., MOSES, M. S. and HOPPE, E. N. 1926. Chromosome behavior and genetic behavior in *Sciara* (Diptera). I. Chromosome behavior in the spermatocyte divisions. — Z. Ind. Abst.- u. Vererb. 42: 237—270.

METZNER, R. 1894. Beiträge zur Granulalehre. I. Kern und Kerntheilung. — Arch. f. Anat. und Physiol. 1894: 309—348.

MICKEY, G. H. 1946. The presence of multiple strands in chromosomes of *Romalea* (Orthoptera). — Amer. Nat. 80: 446—452.

MILLER, D. D. 1939. Structure and variation of the chromosomes in *Drosophila algonquin*. — Genetics 24: 699—708.

MOH, C. C. and NILAN, R. A. 1954. »Short» chromosome — A mutant in barley induced by atomic bomb irradiation. — Cytologia 19: 48—53.

MOORHEAD, P. S. 1954. Chromosome variation in giant forms of *Drosophila montana*. — Univ. Texas Publ. 5422: 106—129.

MORGAN, D. T. JR. 1950. A cytogenetic study of inversions in *Zea mays*. — Genetics 35: 153—174.

MORGAN, T. H., BRIDGES, C. B. and SCHULTZ, J. 1932. The constitution of the germinal material in relation to heredity. — Carneg. Inst. Year Book No. 31: 303—307.

MORRISON, J. W. 1953 a. Chromosome behaviour in wheat monosomics. — Heredity 7: 203—217.

— 1953 b. Pollen formation in pentaploid and near-pentaploid wheat hybrids. — Heredity 7: 419—428.

MORRISON, J. W. and UNRAU, J. 1952. Frequency of micronuclei in pollen quartets of common wheat monosomics. — Canad. J. Bot. 30: 371—378.

MOTA, M. 1952. The action of seed extracts on chromosomes. — Arquivo Patologia 24: 336—357.

MULLER, H. J. 1925. The regionally differential effect of X-rays on crossing over in autosomes of *Drosophila*. — Genetics 10: 470—507.

— 1932. Further studies on the nature and causes of gene mutations. — Proc. Sixth Int. Cong. Genetics vol. 1: 213—255.

— 1940. Bearings of the »*Drosophila*» work on systematics. — The New Systematics, Edited by J. HUXLEY: 185—268.

MULLER, H. J. and HERSKOWITZ, I. H. 1954. Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster*. — Amer. Nat. 88: 177—208.

MÜNTZING, A. 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas 30: 231—248.

— 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at the pollen mitosis. — Hereditas 32: 97—119.

— 1948 a. Cytological studies of extra fragment chromosomes in rye. V. A new fragment type arisen by deletion. — Hereditas 34: 435—442.

— 1948 b. Accessory chromosomes in *Poa alpina*. — Heredity 2: 49—61.

— 1954. Cyto-genetics of accessory chromosomes (B-chromosomes). — Proc. 9th Int. Cong. Genetics, Caryologia, Suppl. 6: 282—301.

MÜNTZING, A. and LIMA-DE-FARIA, A. 1949. Pachytene analysis of standard fragments and large iso-fragments in rye. — Hereditas 35: 253—268.

— and — 1952. Pachytene analysis of a deficient accessory chromosome in rye. — Hereditas 38: 1—10.

— and — 1953. Pairing and transmission of a small accessory iso-chromosome in rye. — Chromosoma 6: 142—148.

NAWASCHIN, S. 1927. Zellkerndimorphismus bei *Galtonia candicans* DES. und einigen verwandten Monokotylen. — B. Deut. Bot. Gesellschaft 45: 415—428.

NEBEL, B. R. 1935. Chromosomenstruktur VI. Ein Ausschnitt. — Züchter 7: 132—136, 155—156.

NEVES, J. B. 1945. Sur l'origine de *Ranunculus dichotomiflorus* LAG. — Bol. Soc. Brot., 2 S, 19: 729—753.

NOVITSKI, E. 1946. Chromosome variation in *Drosophila athabasca*. — Genetics 31: 508—524.

OEHLKERS, F. 1953. Chromosome breaks influenced by chemicals. — Heredity Suppl. 6: 95—105.

O'MARA, J. G. 1939. Observations on the immediate effects of colchicine. — J. Heredity 30: 34—37.

— 1951. Cytogenetic studies on *Triticale* II. The kinds of intergeneric chromosome addition. — Cytologia 16: 225—232.

PATTERSON, J. T. and STONE, W. S. 1952. Evolution in the genus *Drosophila*. — New York. 610 p.

PAVAN, C. 1946. Chromosomal variation in *Drosophila nebulosa*. — Genetics 31: 546—557.

PETERS, J. J. 1946. Cytological effects of sulphanilamide on *Allium Cepa*. — Bot. Gaz. 107: 390—392.

PIZA JR., S. DE T. 1946. Uma nova modalidade de sexo-determinacao no grilo sulamericano *Eneoptera surinamensis*. — Anais da Escola Superior de Agricultura »Luiz de Queiroz» 3: 69—88.

PRAKKEN, R. and LEVAN, A. 1946. Notes on the colchicine-meiosis of *Allium cernuum*. — Hereditas 32: 123—126.

PROPACH, H. 1940. Die Centromeren in der Pollenkornmitose von *Tradescantia gigantea* ROSE. — Chromosoma 1: 521—525.

RANDOLPH, L. F. 1941. Genetic characteristics of the B chromosomes in maize. — Genetics 26: 608—631.

REES, H. 1953. Centromere control of chromosome splitting and breakage. — Heredity Suppl. 6: 235—245.

REGNERY, D. C. 1944. A leucineless mutant strain of *Neurospora crassa*. — J. Biol. Chemistry 154: 151—160.

RESENDE, F. and MANARTE, M. 1951. Physiological changes as the cause of mutations. — Portug. Acta Biol. (A) 3: 219—233.

RHOADES, M. M. 1936. A cytogenetic study of a chromosome fragment in maize. — Genetics 21: 491—502.

— 1938. On the origin of a secondary trisome through the doubling of a half-chromosome fragment. — Genetics 23: 163—164.

— 1940. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. — Genetics 25: 483—520.

— 1950. Meiosis in maize. — J. Heredity 41: 58—67.

— 1952. Preferential segregation in maize. — Heterosis, Edited by J. W. GOWEN: 66—80.

RHOADES, M. M. and VILKOMERSON, H. 1942. On the anaphase movement of chromosomes. — Proc. Nat. Acad. Sci. 28: 433—436.

ROMAN, H. 1947. Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. — Genetics 32: 391—409.

— 1950. Factors affecting mitotic nondisjunction in maize. — Genetics 35: 132.

SANCHEZ-MONGE, E. 1950 a. Two types of misdivision of the centromere. — Nature 165: 80.

— 1950 b. Univalent mechanism and misdivision. — An. Est. Exp. Aula Dei 2: 1—11.

— 1951. The stability of isochromosomes. — An. Est. Exp. Aula Dei 2: 168—173.

SANCHEZ-MONGE, E. and MAC KEY, J. 1948. On the origin of subcompactoids in *Triticum vulgare*. — Hereditas 34: 321—337.

SATINA, S. and BLAKESLEE, A. F. 1935. Cytological effects of a gene in *Datura* which causes dyad formation in sporogenesis. — Bot. Gaz. 96: 521—532.

SAX, K. and MATHER, K. 1939. An X-ray analysis of progressive chromosome splitting. — J. Genetics 37: 483—490.

SCHAEDE, R. 1936. Untersuchungen mit der Nuclealreaktion an Kern und Kernteilung. — Planta 26: 167—192.

— 1937. Anordnung und Gestalt der Chromosomen von *Galtonia candicans*. — Ber. Deutsch. Bot. Ges. 55: 485—492.

SCHRADER, F. 1936. The kinetochore or spindle fibre locus in *Amphiuma tridactylum*. — Biol. Bull. LXX: 484—498.

— 1939. The structure of the kinetochore at meiosis. — Chromosoma 1: 230—237.

— 1944. Mitosis. The movements of chromosomes in cell division. — New York. 110 p.

— 1953. Mitosis. The movements of chromosomes in cell division. — New York. 170 p.

SCHULTZ, J. 1941. The function of heterochromatin. — Proc. VII. Int. Genet. Congr. Edinburgh 1939: 257—262.

SCHULTZ, J. and REDFIELD, H. 1951. Interchromosomal effects on crossing over in *Drosophila*. — Cold Spring Harbor Symp. Quant. Biol. 16: 175—197.

SEARS, E. R. 1944. Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. — Genetics 29: 232—246.

— 1952. Misdivision of univalents in common wheat. — Chromosoma 4: 535—550.

SEARS, E. R. and CAMARA, A. 1950. Un cromosoma dicentrico transmisible en trigo. — Genetica Iberica 2: 239—256.

— and — 1952. A transmissible dicentric chromosome. — Genetics 37: 125—135.

SERRA, J. A. 1949. A cytophysiological theory of the gene, gene mutation and position effect. — Portug. Acta Biol. (A), R. B. GOLDSCHMIDT vol. 1949: 401—562.

SHARP, L. W. 1934. Introduction to Cytology. — New York. 567 p.

— 1943. Fundamentals of Cytology. — New York. 270 p.

SINGLETON, J. R. 1953. Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. — Amer. J. Bot. 40: 124—144.

SMITH, L. 1941. An inversion, a reciprocal translocation, trisomics, and tetraploids in barley. — J. Agric. Res. 63: 741—750.

— 1947. A fragmented chromosome in *Triticum monococcum* and its use in studies of inheritance. — Genetics 32: 341—349.

SMITH, S. G., HUSKINS, C. L. and SANDER, G. F. 1950. Mutations in polyploid cereals. III. The cytogenetics of compactoid wheats. — Canadian J. Res., C, 28: 153—182.

SPARROW, A. H., POND, V. and SPARROW, R. C. 1952. Distribution and behaviour of supernumerary chromosomes during microsporogenesis in a population of *Trillium erectum* L. — Amer. Nat. 86: 277—292.

STADLER, L. J. 1939. Genetic studies with ultraviolet radiation. — Proc. 7th Int. Genet. Congr.: 269—276.

— 1941. The comparison of ultraviolet and X-ray effects on mutation. — Cold Spring Harb. Sympos. Quant. Biol. 9: 168—177.

STEINBERG, A. G. 1936. The effect of autosomal inversions on crossing over in the X-chromosome of *Drosophila melanogaster*. — Genetics 21: 615—624.

STEINBERG, A. G. and FRASER, F. C. 1944. Studies on the effect of X chromosome inversions on crossing over in the third chromosome of *Drosophila melanogaster*. — Genetics 29: 83—103.

STEINEGGER, E. and LEVAN, A. 1947. Constitution and c-mitotic activity of iso-colchicine. — Hereditas 33: 385—396.

STEINITZ-SEARS, L. M. and SEARS, E. R. 1953. Chiasmata and crossing over in a dicentric chromosome in wheat. — Genetics 38: 244—250.

STERN, C. 1936. Somatic crossing over and segregation in *Drosophila melanogaster*. — Genetics 21: 625—730.

STERN, K. G. 1952. Problems in nuclear chemistry and biology. — Exp. Cell Research Suppl. 2: 1—15.

STURTEVANT, A. H. and DOBZHANSKY, TH. 1936. Observations on the species related to *Drosophila affinis*, with descriptions of seven new forms. — Amer. Nat. 70: 574—584.

SWANSON, C. P. 1940. The use of acenaphthene in pollen tube technic. — Stain Techn. 15: 49—52.

— 1947. X-ray and ultraviolet studies on pollen tube chromosomes. II. The quadri-

partite structure of the prophase chromosomes of *Tradescantia*. — Proc. Nat. Acad. Sci. 33: 229—232.

TAN, C. C. 1935. Salivary gland chromosomes in the two races of *Drosophila pseudoobscura*. — Genetics 20: 392—402.

TJIO, J. H. and LEVAN, A. 1950 a. Quadruple structure of the centromere. — Nature 165: 368.

— 1950 b. The use of oxiquinoline in chromosome analysis. — Anales Est. Exp. Aula Dei 2: 21—64.

— 1954. Chromosome analysis of three hyperdiploid ascites tumours of the mouse. — Lunds Univ. Årsskr. (2) 50 (15) Kungl. Fysiogr. Sällsk. H. 65 (15): 1—38.

UNRAU, J. 1950. The use of monosomes and nullisomes in cytogenetic studies of common wheat. — Scientific Agric. 30: 66—69.

UPCOTT, M. 1936. The origin and behaviour of chiasmata. XII. *Eremurus spectabilis*. — Cytologia 7: 118—130.

— 1937. The genetic structure of *Tulipa*. II. Structural hybridity. — J. Genetics 34: 339—398.

WADDINGTON, C. H. 1951. The character of biological form. — Aspects of form, edited by L. L. WHYTE: 43—56.

— 1953. The origin of biological pattern. — New Biology 15: 117—125.

WAGNER, M. N. 1950. The action of X-rays on chromosomes of *Triticum vulgare* HOST. — Genetica Iberica 2: 39—51.

WALTERS, M. S. 1954. A study of pseudobivalents in meiosis of two interspecific hybrids of *Bromus*. — Amer. J. Bot. 41: 160—171.

WALTERS, M. S. and GERSTEL, D. U. 1948. A cytological investigation of a tetraploid *Rhoeo discolor*. — Amer. J. Bot. 35: 141—150.

WARD, C. L. 1952. Chromosome variation in *Drosophila melanica*. — Univ. Texas Publ. 5204: 137—157.

WARTERS, M. 1944. Chromosomal aberrations in wild populations of *Drosophila*. — Univ. Texas Publ. 4445: 129—174.

WATSON, J. D. 1955. Biological consequences of the complementary structure of DNA. — J. Cellular and Comparative Physiology 45 (Suppl. 2): 109—118.

WHITAKER, T. W. and FLORY JR., W. S. 1955. *Tulbaghia violacea* — description, cultural and cytological observations. — Plant Life 2, Herbertia 1955: 64—67.

WHITE, M. J. D. 1954. Animal Cytology and Evolution. — Cambridge. 454 p.

WHITTINGHILL, M. 1948. The effects of methyl-bis (b-chloroethyl) amine upon recombination values in *Drosophila melanogaster*. — Genetics 33: 634.

— 1951. Some effects of gamma rays on recombination and on crossing over in *Drosophila melanogaster*. — Genetics 36: 332—355.

WILSON, E. B. 1931. The distribution of sperm-forming materials in scorpions. — J. of Morphology 52: 429—483.

WITKUS, E. R. and BERGER, C. A. 1944. Veratrine, a new polyploidy inducing agent. — J. Hered. 35: 130—133.

WOLF, B. E. 1950. Die Chromosomen in der Spermatogenese der Dipteren *Phryne* und *Mycetobia*. — Chromosoma 4: 148—204.

ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas 33: 261—296.

— 1948. Chromosome bridges and breaks by coumarin. — Bot. Not. 1948: 376—380.

ÖSTERGREN, G. 1950. Isopycnosis and isopycnotic, two new terms for use in chromosome studies. — Hereditas 36: 511—513.

— 1951. The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. — Hereditas 37: 85—156.

ÖSTERGREN, G. and VIGFUSSON, E. 1953. On position correlations of univalents and quasi-bivalents formed by sticky univalents. — Hereditas 39: 33—50.

Contents

I. Introduction 85

II. Structure and properties of the kinetochore 86

1. The fundamental pattern 86
2. Generality of occurrence of the fundamental pattern 88
3. The division cycle and its implications 93
4. Criteria used in the delimitation of the kinetochore 100
5. Similarities and differences between the kinetochore and the arms. The degree of heteropycnosis 101

III. The genetics of the kinetochore 103

1. The kinetochore as a reversed repeat 103
2. The induction of breakage 105
3. Structural and functional variation 109
4. Chemical composition 115
5. Limitations of the methods used in the location of genes 119

IV. Interrelation and interaction of the kinetochore with other chromosome regions 122

1. The structural evidence. The gradients 122
2. The variation observed within the gradients. The chromosome field 123
3. Non-random distribution of properties along the chromosome body 126
4. The main objections raised against the concept of chromosome field 128
5. The experimental evidence 131

Interaction of the kinetochore with other chromosome regions 131
Interaction of distal regions with other chromosome regions 132
Interaction among other types of chromosome regions 134

V. Evolutionary and morphogenetic implications of the chromosome field 140

Summary 145

Literature cited 149

THE MITOTIC MECHANISM OF TWO MOUSE ASCITES TUMOURS

By *TARVO OKSALA*
WOMEN'S CLINIC, THE UNIVERSITY OF HELSINKI, FINLAND

I. INTRODUCTION

THE transplantable ascites tumours of both the rat and the mouse have opened up certain new avenues for cytological cancer research. The first cytological studies of ascites were made by Japanese investigators on the Yoshida sarcoma of the rat and some other rat tumours (*cf.* MAKINO and KANÔ, 1951; MAKINO and YOSIDA, 1951; MAKINO, 1952 a and b; MAKINO and KANÔ, 1953). One of their main conclusions was that these tumours are characterized by the presence of a certain number of metacentric chromosomes which are absent in the normal chromosome complement of the rat. Recently, however, studies on tissue culture material of the Norway rat (MAKINO and HSU, 1954) have shown that the normal complement of this species, in addition to the acrocentric chromosomes, contains eight autosomal pairs and an X-chromosome which have subterminal or submedian centromeres.

That structurally changed chromosome types may, however, be characteristic of cancer cells was shown by BAYREUTHER (1952) in the hyperdiploid Ehrlich mouse ascites tumour. In this tumour, one very long and one metacentric chromosome, as well as a number of very small fragment-like chromosomes, deviated from the chromosomes of the normal complement of the mouse.

Structural changes in the chromosomes have likewise been described in several ascites tumours by LEVAN and HAUSCHKA (1953 a) and by TJIO and LEVAN (1954). The detailed studies of these authors have further revealed the important fact that each tumour is characterized by a modal chromosome number *s*, which in most cases lies either near the diploid number for the mouse or near the tetraploid number. This and other facts have led the authors to conclude (LEVAN and HAUSCHKA, 1952, 1953 a; LEVAN, 1954) that each tumour is maintained from one transplant generation to another by a *stem line*, whose cells have the exact modal chromosome number *s* and divide by regular mitosis. The great mitotic irregularities which are observed in many cancer cells

occur in cells which — so to say — have been pushed out of the stem line.

The same idea that a certain type of cell with regular mitosis and little variation in chromosome number is responsible for the maintenance of the tumour has been put forward for the Yoshida sarcoma by MAKINO (1951) and MAKINO and YOSIDA (1951).

In addition to the modal chromosome number, other cytological features are characteristic of each ascites tumour. In the words of LEVAN and HAUSCHKA (1953 a, p. 1): »... mitosis in each tumor has its own typical pattern with regard to such properties as the average amount of heterochromatin in the resting nuclei, chromosome size, and state of chromosome contraction. The same holds true for the frequency and kind of aberrations from the normal mitotic course.»

In a number of studies THERMAN and TIMONEN (TIMONEN and THERMAN, 1950; TIMONEN, 1950; THERMAN and TIMONEN, 1950) have attacked the problem of cancer mitosis from an entirely different standpoint. By examining a great number of malignant human tumours from the female genital tract they have come to the conclusion that among the phenomena which distinguish cancer mitosis from normal mitosis the most constant seems to be a shortening in the relative duration of the prophase as compared with the metaphase.

In a further paper (THERMAN and TIMONEN, 1954) it was shown that the ratio of metaphases to prophases (which they termed the Prophase Index) is positively correlated with the occurrence and frequency of multipolar divisions. The first tripolar divisions appear when this index reaches a certain value and their number increases with an increasing index.

The various cytological phenomena in the mouse ascites tumours have been described in great detail, especially by LEVAN and HAUSCHKA (1952, 1953 a and b) and TJIO and LEVAN (1954). In this respect the present paper has little to add. Rather, it is primarily concerned with the growth dynamics of the ascites tumour, and consequently attention has mainly been focused on the mitotic mechanism as revealed by the relative frequencies of the mitotic phases and the various divergent modes of mitosis. One of the chief problems for whose solution the ascites tumours offer new possibilities has been the question of how characteristic the ratios of the different mitotic phases are for a certain type of tumour, and the possible range of variation of their values. This question has attained additional interest since TIMONEN (1956) has found this ratio useful even in clinical cancer diagnosis.

II. MATERIAL AND TECHNIQUE

As material for the present study two mouse ascites tumours were used, *i. e.*, the tetraploid Ehrlich tumour and the hyperdiploid Landschütz I tumour. These were chosen as representing in many ways cytologically opposite types. In a preliminary study, their mitotic mechanism showed a number of different features in addition to the degree of »ploidy».

The animals used were commercial white mice (males) which, however, came from one firm where the strain had been kept for several years. The inoculations were made with ascites suspended in buffered physiological saline (1 : 9) 0,2 ml per mouse.

The preparations were made with acetic-orcein according to the technique presented by LEVAN and HAUSCHKA (1952). The slides were sealed and kept in a refrigerator. For comparison a number of slides were fixed in acetic-alcohol and stained with Feulgen. All the data given in the present study, however, come from the orcein preparations. It may be mentioned that no prefixation treatments were used.

The magnification of the photographs is *ca.* ×1100.

III. CYTOLOGICAL DESCRIPTION

Between the two ascites strains now investigated there exists an important difference which is conspicuous when they are cultivated side by side, and which is of interest when their mitotic mechanism is considered: The growth rate of the tumours is strikingly different. Using the same culture technique, the development of the Ehrlich tumour is considerably more rapid as compared with the Landschütz tumour, which leads to a shorter life span of the animals inoculated with the former strain of tumour. No statistical comparison has been made on this point, but experience has shown that, with the inoculation method described above, the Ehrlich hosts do not, on an average, live longer than 14—15 days from the inoculation, whereas the Landschütz hosts, as a rule, reach the 20th—21st day.

1. Tetraploid Ehrlich tumour

The chromosome numbers in the tetraploid Ehrlich tumour have been determined by LEVAN and HAUSCHKA (1952). The majority of the cells showed the tetraploid number 80, but counts on both sides of this number were also obtained. A few cells with about the double number

as well as with numbers near the diploid number 40 were found. These observations agree with those made on the present material (*cf.* Fig. 3).

In the present study special attention has been paid to *the relative frequencies of the mitotic phases.* As a general observation it may be mentioned that when the tumour ages, the relative frequencies of prophases and anaphases decrease as compared with the frequency of metaphases (Fig. 12). The exact numbers pertaining to this change will be given in the statistical analysis later.

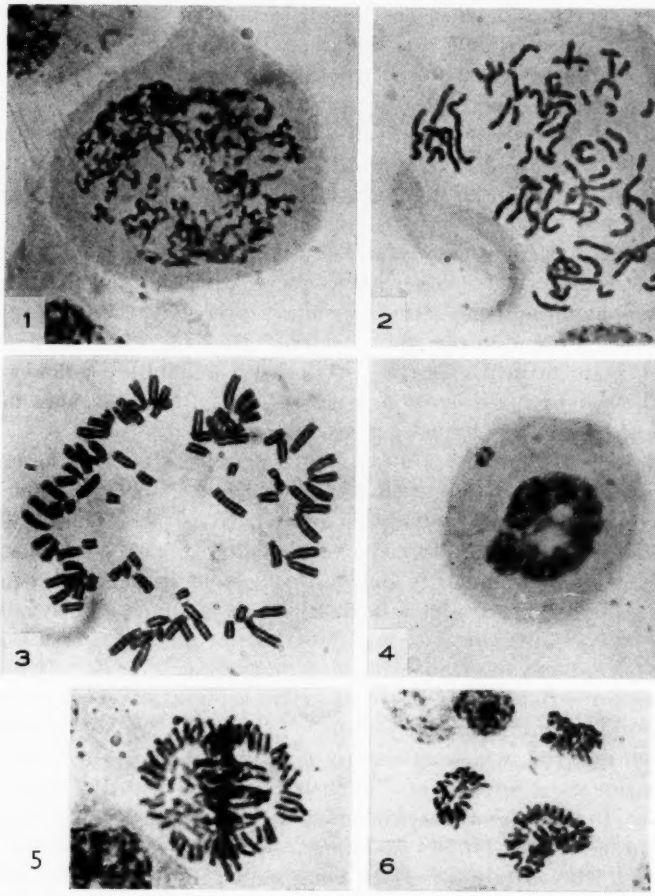
In the tetraploid Ehrlich tumour typical *endomitosis* is very rare, if not absent, as seen in the statistics later on. The number of *endoreduplication* stages is very low, too. This stage has therefore not been scored. According to LEVAN and HAUSCHKA (1953 a), the frequency of endoreduplications is 0,4 % of the total metaphases. At least some of the endoreduplicated cells probably continue to divide by regular mitosis, and these add to the number of polyploid cells.

In every sample of the tetraploid Ehrlich tumour a number of *multipolar divisions* were seen. Tripolar divisions (Figs. 5—6) are most common, but quadripolar divisions also occur. Very occasionally an even higher multipolar division is observed. The total frequency of the multipolar mitoses shows a slight increase as the ascites grows older, especially in tumours more than 10 days old.

It was found in the present material that the frequency of certain types of abnormal mitoses, nuclei, and cells greatly increases with the age of the ascites. The most important of these phenomena (*cf.* LEVAN and HAUSCHKA, 1953 a) is the increase in the number of *polyploid interphases as well as polyploid division stages.* These owe their origin to several different processes.

Another typical phenomenon which becomes more frequent as the ascites grows older is *the reversion of the chromosomes to the interphase or pycnotic state directly from any mitotic stage* (abortive mitosis). This may happen at prophase but is apparently more common at metaphase. When the metaphase plate forms a ring, peculiar ring-shaped interphase nuclei (Fig. 4) result. An anaphase with sticky bridges between the groups may also go into interphase, and thus give rise to a dumb-bell-shaped nucleus, a configuration which in the earlier literature was often interpreted as an amitotic stage. Most of these phenomena perhaps precede the disintegration of the cell, but the possibility naturally remains that a few such cells are viable, and have doubled their chromosome number in the process.

Old ascites is further characterized by the occurrence of *cells with*



Figs. 1—6. Tetraploid Ehrlich tumour. — Fig. 1. Early prophase. — Fig. 2. Late prophase. — Fig. 3. Metaphase. — Fig. 4. Cell with a ring-shaped interphase nucleus and a micronucleus. — Fig. 5. Tripolar metaphase. — Fig. 6. Tripolar anaphase.

more than one nucleus. These also arise in several ways. After a multipolar division of the nucleus the cell itself may fail to divide, and thus obtain several nuclei. [HIRONO (1951) has studied the living mitosis in the Yoshida sarcoma of the rat and found that after a tripolar mitosis the resulting cells may fuse. According to HIRONO, nuclear fusions also

occur. The same phenomenon has been described by NAKAHARA (1953) in living ascites cells. Thus, fusions and restitutions may also add to the number of multinucleate or polyploid cells.]

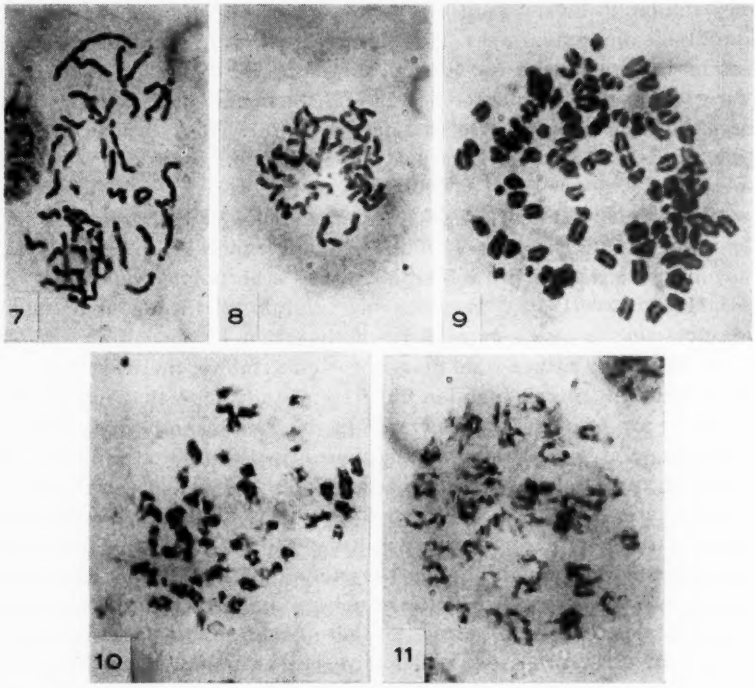
In those cases in which two or three nuclei in one cell undergo mitotic divisions, these are strictly synchronized in their mitotic cycle. LEVAN and HAUSCHKA (1953 b) have studied the cytology of lymphosarcoma 6C3HED and found that in this strain many of the nuclei break up into several micronuclei at telophase, and these micronuclei are synchronized in their mitosis. In the present material a considerable number of cells were observed in which two or three nuclei were in prophase. Synchronized metaphase stages seem to be much rarer; only three indisputable cases being found in the whole material. Division of several nuclei in one cell may in turn give rise to cells with double the number of nuclei. The synchronized divisions occur so rarely, however, that they are more of theoretical interest.

In some cells *the nuclei begin to grow in size*; these nuclei are much more lightly stained than the other nuclei. Such a nucleus seems to swell continuously, often reaching an enormous size. The nucleoli also enlarge. In old ascites (11—15 days) these cells become more frequent, but vanish when the tumour is inoculated into a new mouse. This process of swelling is not considered further in the present paper. Obviously it does not represent typical endomitosis. Possibly it is one of the expressions of the necrotic tendencies in an old tumour.

In every series of slides made of an ascites sample, a few cells showing more or less pronounced *c-mitosis* (*cf.* LEVAN, 1954) were found. The scored material, which admittedly does not extend beyond the 7th day, does not show any increase in their frequency with the aging of the tumour. In the extreme cases in which the chromosomes lie scattered around the cell there follows an interphase with a large number of micronuclei. Obviously most of the c-mitotic stages precede the death of the cell.

2. Hyperdiploid Landschütz I tumour

The chromosomal conditions of the hyperdiploid Landschütz I and II tumours and of the hyperdiploid Ehrlich tumour have been analysed in great detail by TJIO and LEVAN (1954). They present convincing evidence for the view that the two Landschütz tumours are actually sublines of the hyperdiploid Ehrlich tumour, differing only in minor points from this. In regard to chromosome numbers, the Landschütz



Figs. 7—11. Hyperdiploid Landschütz I tumour. — Fig. 7. Middle prophase. — Fig. 8. Late prophase. — Fig. 9. C-mitosis with over 80 chromosomes. — Fig. 10. Endometaphase. — Fig. 11. Endoanaphase.

tumours have a very sharp peak at 46 chromosomes, 90 % of all cells having this number. The same chromosome types deviating from the normal mouse chromosomes, described by BAYREUTHER (1952) in the Ehrlich tumour, have been found by TJIO and LEVAN in the two Landschütz tumours. The only difference is that a deletion seems to have occurred in BAYREUTHER's A chromosome. According to TJIO and LEVAN, both endomitosis and endoreduplication occur in these two tumour strains. The present study bears out these observations (*cf.* Figs. 7—11).

The present analysis shows that the hyperdiploid Landschütz I tumour is in many respects the direct counterpart of the tetraploid Ehrlich tumour.

The relative frequencies of the mitotic phases, which are initially

very similar to those found in the young Ehrlich tumour, change profoundly as the tumour ages. This change proceeds in the opposite direction as compared with the Ehrlich tumour: The frequency of the prophases increases considerably, while the frequency of metaphases (and anaphases) decreases correspondingly (Fig. 13). The character and degree of this change is described in connection with the statistical analysis.

Another difference is the frequent occurrence of *endomitotic stages* (Figs. 10—11) in the Landschütz I tumour, which are practically absent in the tetraploid Ehrlich strain. It has been pointed out by LEVAN and HAUSCHKA (1953 a) that the delimitation of the different endomitotic stages is much more difficult than that of the mitotic phases. Even allowing a rather wide margin for uncertainty, the frequencies of the endomitotic phases found in the present study show that endomitotic processes are of common occurrence in this tumour, especially at later stages (see Table 1). It may be mentioned in passing that a number of cells with two nuclei in synchronized endomitosis were observed.

LEVAN and HAUSCHKA (1953 a) have used the frequency of cells with double the modal chromosome number (2*s*) to indicate the frequency of endomitosis. Although in regard to endomitosis the present method of scoring may give results which are not quite exact, their method likewise contains sources of error, as there occur in the tumour cells a number of other processes, which give rise to polyploid nuclei, apart from endomitosis. A number of these have been considered above, in connection with the Ehrlich tumour.

The third difference between mitosis in the tetraploid Ehrlich tumour and in the Landschütz tumour is the occurrence of *multipolar divisions* in the former and their almost total absence in the latter. Only very occasionally has a solitary multipolar division been observed in the Landschütz tumour (Table 1).

As the Landschütz ascites grows older, a number of phenomena similar to those in the aging Ehrlich ascites are seen. The anaphases become sticky and often contain bridges. The tetraploid (2*s*) divisions (Fig. 9) increase in number. Large interphase nuclei or cells with several nuclei also become more frequent. The lightly stained giant cells, which are so frequent in the old Ehrlich tumour, are much rarer in the Landschütz tumour. The frequency of c-mitosis (Fig. 9) is the same in the two tumours (Table 1).

IV. STATISTICAL ANALYSIS

In the cytological description above, it has been mentioned that the ratios of the frequencies of the mitotic phases change in a certain way with the development of the tumour, and that these changes are strikingly dissimilar in the two tumour strains investigated. As this question is pertinent to the present study, a number of exact scorings have been performed to ascertain the quality and quantity of these changes. However, a complete analysis of the variation of the mitotic phases during the whole development of the tumours has not been made. Instead, a comparison has been made between the incipient and the far-developed tumour.

1. Methods

The material consists of six samples of 3-day-old tumours and the same number of samples of 7-day-old tumours of the Ehrlich and the Landschütz ascites strains. Each sample comes from a different host. Ascites younger than 3 days old has not been investigated, since it is probable or at least possible that such ascites fluid contains cells from the original inoculum. These would lend a false character to a tumour, which in fact is only just beginning to grow. In order to let the ascites tumour develop undisturbed, only one sample has been taken from each mouse. The inoculum, which came from 7- or 8-day-old tumours, was practically always from a different animal; individual variation of the inoculated samples is thus not excluded. Since the inoculation technique, which has been described earlier (p. 163), is rather crude, it has been impossible to determine the actual number of cells inoculated. Consequently, quantitative differences are also bound to exist between the inoculums. This in turn may cause differences in the growth of the tumours, as can be concluded from the studies of KLEIN (1951). (Since the host animals are not genetically completely identical, we have here a third factor which may affect the growth of the tumours.)

These sources of error account for the fact that samples of the same tumour and the same age in some cases show statistically significant differences in the relative frequencies of the mitotic phases. Some of the samples of the two groups overlap, although they are as a whole typically different. The variations in the different samples of the same age and the same tumour may be assumed to level each other out, and therefore in the following attention has in each group been focused on the total material obtained from the six mice.

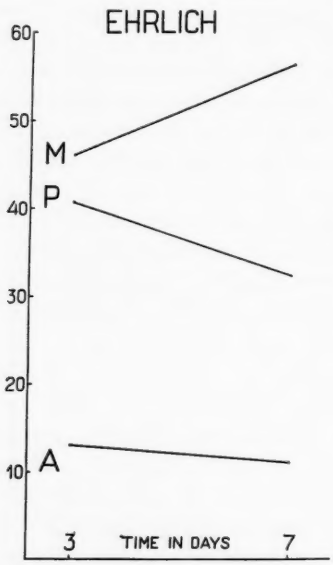


Fig. 12. Ehrlich tumour. The change in the frequencies of metaphases (M), prophases (P), and anaphases (A) (as percentages from total of these phases).

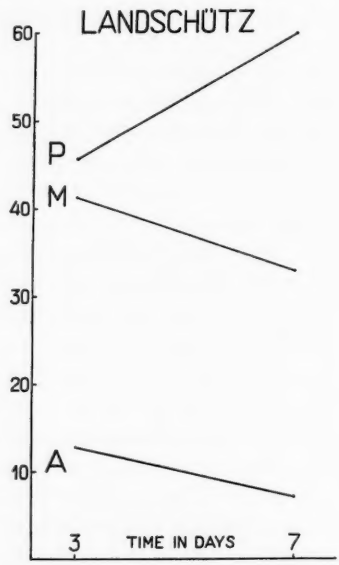


Fig. 13. Landschütz tumour. The change in the frequencies of prophases (P), metaphases (M), and anaphases (A) (as percentages from total of these phases).

The scoring of the mitotic phases has been done by going over each slide with an immersion objective and ×15 eye-pieces in a series of parallel lines, and making a note of all prophases, metaphases, anaphases, multipolar divisions, so-called c-effects, and endomitotic stages. The criteria used in the delimitation of the stages are obvious from Figs. 1—11. The only stages excluded from the count are thus the telophases and interphases. The total of the cells counted from each mouse is 300—500. As a rule, 4—8 slides of each ascites sample — depending on the frequency of the mitotic stages — have been examined in this manner.

The original material thus obtained, on which the following discussion is based, is presented in Table 1. Since the relative frequencies of the mitotic phases in it are especially pertinent, these frequencies are given as percentages in Table 2. In addition, the relative frequencies are given as indices as follows: the ratio metaphases/prophases (M/P), the

TABLE 1. *Number of prophases, metaphases, anaphases, multipolar mitoses, c-mitoses, and endomitoses in 3- and 7-day-old samples of Landschütz and Ehrlich ascites tumours (total 24 mice).*

	Mouse No.	Pro-phases	Meta-phases	Ana-phases	Multip. mitoses	c-mitoses	Endo-mitoses	Total
Ehrlich 3 days	1	151	134	56	9	1	—	351
	2	186	205	55	5	4	2	457
	3	164	184	51	2	1	1	403
	4	136	168	42	2	—	—	348
	5	136	168	35	8	7	—	354
	6	124	159	48	3	1	—	335
	Total	897	1018	287	29	14	3	2248
Ehrlich 7 days	1	120	187	40	2	1	—	350
	2	130	220	34	5	8	1	398
	3	137	231	47	3	1	1	420
	4	111	203	26	5	4	—	349
	5	109	211	48	4	—	1	373
	6	111	226	36	3	—	—	376
	Total	718	1278	231	22	14	3	2266
Landsch. 3 days	1	150	178	56	1	7	1	393
	2	157	151	27	1	2	8	346
	3	161	152	44	3	1	7	368
	4	201	178	71	—	3	10	463
	5	213	170	63	1	2	9	458
	6	165	125	34	2	4	12	342
	Total	1047	954	295	8	19	47	2370
Landsch. 7 days	1	240	196	32	1	3	30	502
	2	254	147	34	1	2	10	448
	3	208	107	29	—	4	11	359
	4	241	118	16	—	—	21	396
	5	219	99	30	—	7	13	368
	6	228	95	23	—	4	29	379
	Total	1390	762	164	2	20	114	2452

ratio metaphases/anaphases (M/A), and the ratio prophases/anaphases (P/A). The same data can be seen from the diagram in Fig. 14.

2. The Ehrlich tumour

The ratio metaphases/prophases ranges on the 3rd day from 0,89 to 1,28, being 1,14 for the total material. The values for the 7th day vary

TABLE 2. *Percentages of prophases, metaphases, and anaphases from the total of these mitotic phases and the ratios metaphases/prophases (M/P), metaphases/anaphases (M/A), and prophases/anaphases (P/A). (The same samples as in Table 1).*

	Mouse No.	Pro-phases	Meta-phases	Ana-phases	Total no. of cells	M/P	M/A	P/A
Ehrlich 3 days	1	44,28	39,30	16,42	341	0,89	2,39	2,70
	2	41,70	45,97	12,33	446	1,10	3,73	3,38
	3	41,10	46,12	12,78	399	1,12	3,61	3,22
	4	39,31	48,55	12,14	346	1,24	4,00	3,24
	5	40,12	49,56	10,32	339	1,24	4,80	3,89
	6	37,46	48,04	14,50	331	1,28	3,31	2,58
	Total	40,74	46,23	13,03	2202	1,14	3,55	3,13
Ehrlich 7 days	1	34,58	53,89	11,53	347	1,56	4,68	3,00
	2	33,85	57,29	8,86	384	1,69	6,47	3,82
	3	33,01	55,66	11,33	415	1,69	4,92	2,92
	4	32,65	59,71	7,64	340	1,83	7,81	4,27
	5	29,62	57,34	13,04	368	1,94	4,40	2,27
	6	29,76	60,59	9,65	373	2,04	6,28	3,08
	Total	32,24	57,39	10,37	2227	1,78	5,53	3,11
Landsch. 3 days	1	39,06	46,36	14,58	384	1,19	3,18	2,68
	2	46,87	45,07	8,06	335	0,96	5,59	5,82
	3	45,10	42,58	12,32	357	0,94	3,46	3,66
	4	44,67	39,55	15,78	450	0,89	2,51	2,83
	5	47,76	38,12	14,12	446	0,80	2,70	3,38
	6	50,93	38,58	10,49	324	0,76	3,68	4,85
	Total	45,60	41.55	12,85	2296	0,91	3,23	3,55
Landsch. 7 days	1	51,28	41,88	6,84	468	0,82	6,13	7,50
	2	58,39	33,79	7,82	435	0,58	4,32	7,47
	3	60,47	31,10	8,43	344	0,51	3,69	7,17
	4	64,26	31,47	4,27	375	0,49	7,38	15,07
	5	62,93	28,45	8,62	348	0,45	3,30	7,30
	6	65,89	27,46	6,65	346	0,42	4,14	9,91
	Total	60,02	32,90	7,08	2316	0,55	4,65	8,48

from 1,56 to 2,04. The value for the total material is 1,78, showing a clear difference from the situation obtaining on the 3rd day. During the development of the tumour the frequency of the metaphases has thus greatly increased. The difference between these two statistics is highly significant (χ^2 with 1 degree of freedom = 48,58; $P < 0{,}001$).

The ratio metaphases/anaphases varies considerably more than the previous index both on the 3rd and on the 7th day. This depends on the small absolute number of anaphases, which may easily cause random variation between the different samples. The same explanation applies to the fact that the values for the 3rd and the 7th day partly overlap. When the whole material is considered, in which the random differences are levelled out, the difference between the old and the young ascites is, however, clear: on the 3rd day M/A is 3,55 on the 7th day 5,53. The difference is highly significant ($P < 0{,}001$), showing that the number of metaphases as compared with anaphases increases clearly during the development of the tumour.

The ratio prophases/anaphases. — In contradistinction to the other two ratios, this ratio is practically the same on the 3rd (3,13) as on the 7th day (3,11). This depends on the fact that the frequency of both the prophases and the anaphases has dropped equally from the 3rd to the 7th day (the frequency of prophases has decreased by 20,9 %, the frequency of anaphases by 20,4 %).

3. The Landschütz I tumour

The ratio metaphases/prophases. — The values for the 3rd and the 7th day overlap slightly. When the total material is considered the value of M/P changes in four days from 0,91 to 0,55. The difference is statistically highly significant ($P < 0{,}001$). The number of prophases thus shows a clear increase as compared with the metaphases. The direction of the change is thus the opposite of that found in the Ehrlich tumour.

The values for *the ratio metaphases/anaphases* show, as in the Ehrlich tumour, a great dispersal in both the younger and the older tumours. The explanation in this case, too, is the small number of anaphases. The overlapping of the values for the 3rd and the 7th day is even more considerable than in the Ehrlich tumour. This is also seen from the fact that on aging of the tumour the ratio M/A for the total material increases less than in the Ehrlich tumour (from 3,23 to 4,65). The difference between the younger and the older tumour is clear and statistically highly significant in this case also ($P < 0{,}001$).

The ratio prophases/anaphases. — Since between the 3rd and the 7th day the number of prophases increases as compared with the metaphases, and the number of metaphases increases as compared with the anaphases, it is obvious that the increase in the ratio P/A must be considerable (from 3,55 to 8,48). No overlapping between the samples of

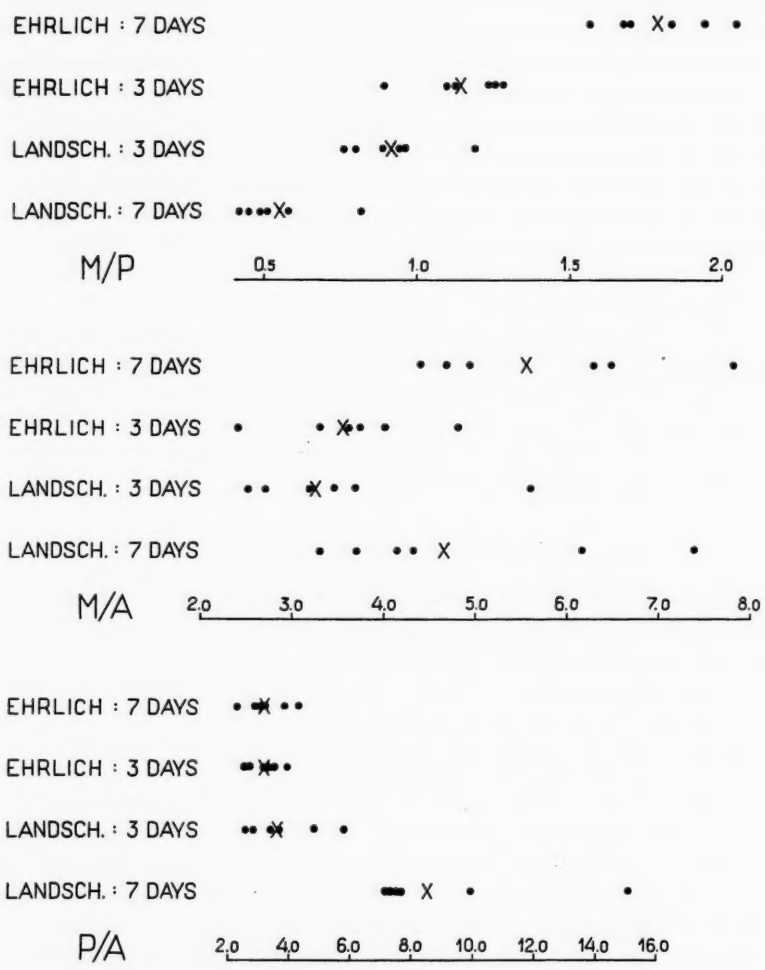


Fig. 14. The ratios metaphases/prophases (M/P), metaphases/anaphases (M/A), and prophases/anaphases (P/A) in 3- and 7-day-old Ehrlich and Landschütz I tumours. Dots indicate the ratios of individual samples, crosses those of the totals in each series of samples.

different age occurs. The situation is completely different from that established for the Ehrlich tumour.

4. Comparison of the two tumours

If we compare the Ehrlich and the Landschütz tumours on the 3rd day from inoculation (Figs. 12—14), it is seen that the relative frequencies of the mitotic phases are very similar. The percentage of anaphases is practically the same. The percentages of prophases and metaphases are very near each other, the former being slightly in excess in the Landschütz tumour, the latter in the Ehrlich tumour. The ratio M/P is 0,91 in the Landschütz tumour; in the Ehrlich tumour it is 1,14. Although the difference between these values is not great, it is statistically highly significant ($P < 0{,}001$). The two series of samples overlap only slightly, and the ratio M/P in the two tumours is such that it clearly indicates the direction of the later development. It seems probable (see Figs. 12—13) that at stages earlier than 3 days the situation would be the same in the two tumours, the ratio M/P probably being near 1,0. However, tumours younger than 3 days old have not been examined, as the ascites fluid on the first and second day is too meagre. Moreover, their analysis would be confused by the fact — stressed earlier (p. 169) — that such ascites must contain a considerable number of old cells.

In a far-developed case (on the 7th day) the situation has completely changed. In the Ehrlich tumour the stage prevailing in frequency is the metaphase, in the Landschütz tumour the prophase. The difference in distribution is highly significant ($P < 0{,}001$). Their development has thus been divergent. The frequency of the anaphases has decreased in both tumours, in the Landschütz, however, more than in the Ehrlich tumour.

V. INTERPRETATION OF THE RESULTS

1. Normal mitoses

The statistical analysis above gives an exact idea of the differences in the relative frequencies of the mitotic phases prevailing on the one hand between the Ehrlich and the Landschütz tumours, and on the other hand between the different developmental stages of one and the same tumour. Two questions now arise: What do these data disclose about the difference in the mitotic mechanism of these tumours? And, secondly, what are the dynamics of tumour development?

In cytology it is regarded as self-evident that in a growing tissue or in a tissue culture a mitotic stage which occurs abundantly is of longer duration than one which is scarce. If a certain phase becomes clearly more frequent at the same time as another becomes correspondingly rarer, this implies that the duration of the former increases relative to the latter. This line of thought is justified, however, only as long as all the mitoses continue normally from prophase until telophase without any cells going directly into interphase or becoming necrotic from some stage earlier than telophase. If the course of mitosis is often stopped at a certain stage, the later mitotic stages are correspondingly absent from the statistics. Such a decrease in their frequency naturally does not reflect any shortening in their duration.

This ought to be borne in mind when attempting an interpretation of the changes in the relative frequencies of the mitotic stages in the ascites tumours, since, as was mentioned earlier (p. 164), abortive mitoses are typical and relatively common in aging tumours.

This obviously affords the best explanation of the decrease in the percentage of anaphases which is noticeable between the 3rd and the 7th day in both the tumours now studied, since the possibility seems definitely to be excluded that the anaphase movement of the chromosomes is more rapid in the older than in the young tumour. In an incipient tumour all the mitoses take a normal course from prophase to telophase. Later on, however, some of the mitoses which have reached the metaphase do not enter the anaphase, but pass directly over into interphase or become pycnotic. Direct observation (p. 164) shows, in fact, that this is the case.

In the Ehrlich tumour the number of anaphases decreases only relative to the metaphases, while the ratio P/A remains unchanged. This shows that on aging of the tumour the prophase stage has become shorter even absolutely. Otherwise the decrease in the number of anaphases would have led to an increase of the ratio P/A. It is possible and even probable that the metaphase, too, is somewhat lengthened in an aging tumour, as in the abortive metaphases the chromosomes remain longer than normally in the metaphase plate before passing into interphase or into a pycnotic state through some kind of telophase. Such abnormal mitoses cannot, however, be the only factor responsible for the increase in the percentage of metaphases, and of the ratio M/P, since, as already mentioned, this ought to result in an increase of the ratio P/A, too.

The most plausible, and therefore the almost unavoidable, conclusion

thus remains that in the Ehrlich tumour at its most intensive growth period, the chromosomes when passing from interphase to prophase remain in the latter a relatively short time and rapidly form the metaphase plate. In theory such a shortening might have the following results. Either the metaphase is lengthened proportionately to the shortening of the prophase; in this case the mitosis as a whole would retain its former duration. Or the metaphase remains unchanged, which results in the shortening of the duration of the mitosis. A choice between these two alternatives is difficult, since in an aging tumour a new independent phenomenon, which actually increases the duration of the metaphase, comes into play. This consists of a tendency to abortive mitoses, which especially affects the metaphases, blocking the course of the mitotic events at this stage, or possibly in less extreme cases slowing it down.

In short, a characteristic feature in the Ehrlich tumour is a tendency for the prophase to become shortened. In the opinion of the present author, this is most adequately explained by assuming that the formation of the spindle occurs earlier than usual. The cycle of polarity is accelerated in the series of successive mitoses. We thus have here a phenomenon which has been called by the present author *precocity of polarity* (OKSALA, 1954).

In its development the Landschütz tumour represents the opposite of the Ehrlich tumour. The only feature common to them both is a decrease in the percentage of anaphases. In both these tumours this is reflected by an increase in the ratio M/A. However, in the Landschütz tumour the increase in the ratio P/A is even higher; this increase is totally absent in the Ehrlich tumour. This situation is caused by the conspicuous increase in the percentage of prophases in the Landschütz tumour, a phenomenon visible also in the clear decrease of the ratio M/P.

Since abortive mitoses also occur in the Landschütz tumour, as mentioned earlier (p. 168), the decrease in the percentage of anaphases is accounted for. Now, it might be thought that the decrease in the frequency of metaphases relative to prophases could be explained along similar lines by assuming that on aging of the tumour a number of prophases become abortive. Since on the 3rd day the percentage of metaphases relative to prophases is 91, while on the 7th day it is only 55, this would mean that on the 7th day 40 % of the prophases are blocked without entering metaphase. This possibility is, however, out of the question. Although a proportion of the prophases — and possibly a greater proportion than in the Ehrlich tumour — pass directly from

prophase to interphase or become pycnotic, this cannot apply to anywhere near half of the prophases. On the contrary, beautiful, well-developed prophases are characteristic of the Landschütz tumour. Neither does the microscopic picture reveal any large-scale blocking of the mitoses at prophase. Consequently, the most probable explanation of the decrease in the ratio M/P is that on aging of the tumour the prophase shows a tendency to become longer. It is naturally true that a decrease in the ratio M/P might be explained by assuming that the metaphase stage becomes shorter, while the prophase remains unchanged. Such an explanation would necessitate the assumption of abnormally rapid spindle activity, which, however, is not suggested by any of the observations made on the Landschütz tumour.

In the foregoing discussion, *the conclusion has thus been reached that during the growth of the two tumours now under consideration their mitotic mechanisms develop in different directions. In the beginning the duration of the prophase and metaphase is about the same in both the Ehrlich and the Landschütz tumour. Soon, however, the Ehrlich tumour shows a tendency for the prophases to become shortened, while in the Landschütz tumour the prophases become longer. The Ehrlich tumour is thus characterized by the precocity of the spindle. In the Landschütz tumour the formation of the spindle is retarded, and the situation is characterized by the opposite state of affairs to precocious spindle formation.*

These features agree well with the observations (p. 163) concerning the growth rate of these tumours. The Ehrlich tumour grows quicker than the Landschütz tumour; it produces more cells in the same time and gives rise to earlier death of the host animal.

The occurrence of certain abnormal cell types and mitotic types, which have been briefly described earlier, bears out what has been suggested above concerning the difference in their mitotic mechanisms. Of these, only endomitoses and multipolar mitoses will be discussed.

2. Endomitoses

As has been mentioned above, endomitoses are practically lacking in the Ehrlich tumour, but are always present, although their percentage is not high, in the Landschütz tumour. The exact scorings fully bear out this impression. The Ehrlich tumour contains very few, if any, typical endomitoses among the 2000 scored cells, either on the 3rd or on the 7th day of tumour development. In the Landschütz tumour endo-

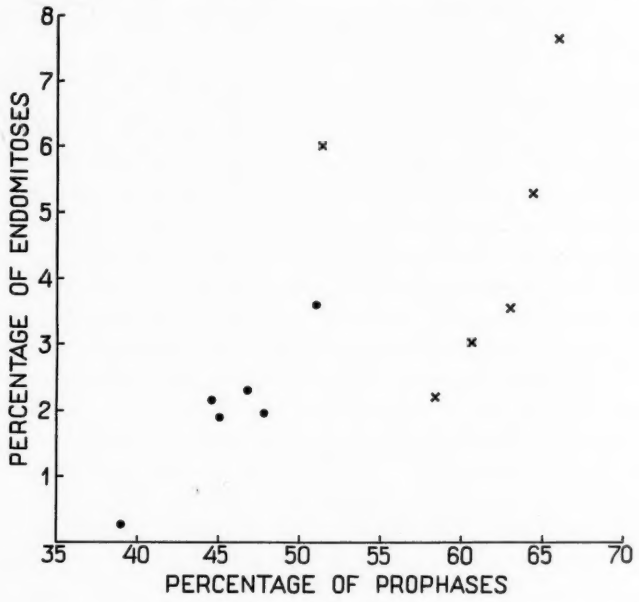


Fig. 15. Landschütz I tumour. Percentage of endomitoses (from total of polarized mitotic phases) plotted against percentage of prophases (from total of scored mitotic phases). Dots indicate 3-day-old samples, crosses 7-day-old ones.

mitoses constitute a noticeable proportion of the total contingency of the cells scored: on the 3rd day 1,98 %, and on the 7th day 4,65 %. (It may be stressed that only quite certain and typical endomitoses have been included.) If the frequency of this mitotic type, which is characterized by the absence of polarization, is given as a percentage of the polarized mitotic stages (normal metaphases and anaphases + multipolar mitoses) the corresponding percentages are 3,74 and 12,29. The incidence of endomitoses is thus considerably higher in an old than in a young Landschütz tumour. The difference is statistically highly significant ($P < 0{,}001$).

Hence, it seems that the tendency to endomitoses is correlated with the tendency of the prophases to become longer, *i. e.*, with the weakening of the polarity. This is seen from two facts: Firstly in a negative way, in that the Ehrlich tumour, which is characterized by strong polar activity (a short prophase), contains practically no endomitoses. And positively, in that the frequency of the endomitoses in the Landschütz

tumour is high and, more particularly, increases with the lengthening of the prophase on aging of the tumour.

The situation can be analysed in greater detail by considering each of the Landschütz tumour samples separately. In Fig. 15 the frequency of prophases, given as a percentage of the total of normal mitotic phases, has been plotted against the percentage of endomitoses from total polarized phases in each of the twelve Landschütz mice studied. It is seen that a clear case of positive correlation ($r = 0{,}714$; $P < 0{,}01$) is present.

The lengthening of the prophase in the normal mitoses and the increase in the frequency of endomitoses thus seem to go hand in hand and are accordingly most probably causally connected. If polarity is present and the spindle is formed in the usual manner, endomitosis is naturally excluded. If, again, the prophase is long and the polarity is overdue or wholly missed out, one prerequisite for the endomitotic process is fulfilled. Naturally this is not the only prerequisite for endomitosis. By no means every lengthened or abortive prophase leads to endomitosis. On the contrary, observations indicate that a nucleus about to undergo endomitosis has embarked on this course directly from interphase. But, since the occurrence of long prophases and of endomitoses is correlated, it is probable that they have a common basis which, depending on other determinants, leads to the one or the other alternative.

3. Multipolar mitoses

Multipolar mitoses are relatively scarce in the present material (Table 1). In the Landschütz tumour they are very infrequent, being obviously rare exceptions. They can be said to be characteristic of the Ehrlich tumour, although even in this their frequency is low. When considering the whole material, the proportion of multipolar mitoses is 1,81 % in the Ehrlich tumour, and 0,46 % in the Landschütz tumour, taken from the number of bipolar mitoses (metaphases and anaphases). The difference is statistically highly significant ($P < 0{,}001$).

As a general observation it has been mentioned earlier (p. 164) that in the Ehrlich tumour the incidence of multipolar mitoses is highest in the old tumours. This is not seen, however, from the present material, which does not contain samples from tumours more than 7 days old. Their incidence does not show a clear difference between the 3-day-old and the 7-day-old samples. In the former the percentage (again in relation to the number of bipolar mitoses) is even somewhat higher (2,22 %) than four days later (1,46 %). The difference is not, however, significant

($P > 0{,}1$). In addition, it is possible that a proportion of the multipolar mitoses in the 3-day-old samples represents cells of the old tumour which have come with the original inoculum, and have not had time to disintegrate. Such cells would thus not be the products of the stem line of the new host. However, there is no definite proof for this view.

In any case, it is noteworthy that multipolar mitoses are much more frequent in the Ehrlich than in the Landschütz tumour. The explanation for this fact is to be found along the same lines on which the whole previous interpretation has been based. The exceptional activity of the polarity in a developing Ehrlich tumour implies that the rate of division of the centrosomes is rapid. Obviously sometimes the division of one or both the centrosomes is precocious and thus takes place even before polarization arises in the cell or immediately at its onset. Such mis-timing results in the formation of a tripolar or a quadripolar mitosis.

Accordingly, it seems that the occurrence of multipolar mitoses is somehow correlated with the shortened prophase and with the precocity of polarity. A corresponding correlation — although much more pronounced — has been found by THERMAN and TIMONEN (1954) in a number of solid human tumours. And a similar correlation has also been described by HSU (1954) in the tissue culture of a human cervical carcinoma.

VI. STEM LINE AND THE GROWTH OF THE ASCITES TUMOUR

In addition to normal mitoses, the two ascites tumours now studied show — as do most other ascites tumours — a high incidence of exceptional mitotic types and as a result cells deviating from the normal. The origin of some of these has been explained above, and certain of the processes have been analysed causally also. Let us now sum up what is known of the succession and interdependence of the different mitotic processes and the resulting cell types on the basis of the present cytological observations.

A sound starting point for such a discussion is provided by the concept of the *stem line* put forward by LEVAN and HAUSCHKA (1953 a; *cf.* also LEVAN, 1954; MAKINO, 1951; MAKINO and YOSIDA, 1951). Since in each new host the ascites tumour begins anew and goes through a similar growth process, producing on aging exceptional mitotic types and cell types in definite ratios, characteristic of each tumour, its growth must be based on a number of identical cells possessing similar growth capacities. It is these cells which constitute the stem line. The stem line

concept has much in common with WEISMANN's well-known germ line (*cf.* LEVAN, 1954, pp. 55—58). The germ line produces a new soma in each generation, the stem line a new tumour in each new host. Neither of them changes itself, but retains its continuity from one generation to another (in the germ line from one individual to another, in the stem line from one tumour generation to another). However, we must beware of drawing the analogy between these concepts too far.

The stem line, accordingly, will be most abundantly represented in a young tumour after the non-dividing cells, which have come in the inoculum, have disintegrated, and new cells which have been pushed out of the stem line are still scarce. In the present material the 3-day-old samples contain relatively most of the stem line cells. Naturally even these samples do not represent the pure stem line, since even at this stage a number of cells have been pushed out of it and other atypical cells may have come with the old tumour. However, the majority of the cells certainly represent the stem line.

In the papers of LEVAN and HAUSCHKA the stem line has been defined as consisting of cells which divide normally and which have the modal number of chromosomes characteristic of each particular tumour. The present study enables a further characteristic to be added to these, *viz.* the relative frequencies of the mitotic phases. In the foregoing (p. 175) the relative duration of the different phases in the two tumours studied was given for the stage (the 3rd day) when the tumours mainly consist of stem line cells. It is an interesting fact that the two tumours show little difference in this respect. And, as has been mentioned above, even this difference would probably be absent from samples less than 3 days old; such samples, however, have not been available owing to technical difficulties. In any case, the important observation was made that in the 3-day-old samples of both the Ehrlich and the Landschütz tumours the prophase and the metaphase are of about the same duration. In this, as in other respects, the stem line behaves in a »normal» manner, resembling any other intensively growing tissue.

This period of »normal» growth is short, however, and before long deviating types of cells and mitotic processes appear, and, interestingly enough, on their production each tumour attains a characteristic direction of development as the situation on the 7th day shows. This has been brought about in the following way: While the stem line maintains itself, cells are continuously being pushed out of it. These cells are devoid of the capacity for growth and division of the stem line cells, but possess new capacities which lead to more or less abnormal processes.

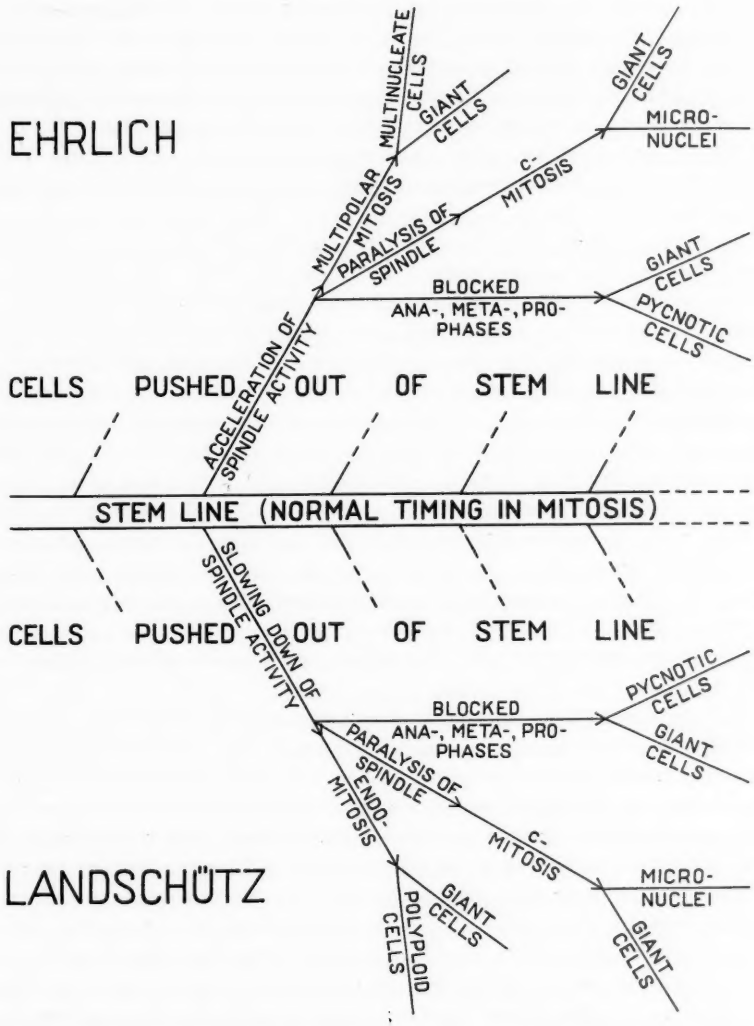


Fig. 16. Mitotic behaviour of cells pushed out of the stem line in the Ehrlich and the Landschütz tumours. Further explanations in the text.

And the longer growth continues, the higher is the incidence of these abnormal cells relative to the frequency of the stem line cells. Thus the features characteristic of each tumour are continuously strengthened. If we make the obviously justified assumption that features deviating least from the course of normal mitosis are primary in this process of differentiation, and that the more gross deviations are secondary, we can reconstruct the development of the two tumours as follows. (Fig. 16 illustrates this process.)

The cells pushed out of the stem line in the Ehrlich tumour show a precocity of polarity, *i. e.*, a shortened prophase. If the interphase likewise becomes shorter — which is possible, although so far unproved — and if several such mitoses succeed one other, the rapid growth of the tumour is accounted for. As a secondary phenomenon this process is accompanied by precocious division of the centrosomes, which leads to multipolar divisions. This in turn results, in some cases at least, in the formation of multinucleate or exceptionally large (polyploid) cells. In an older tumour another factor directly opposed to this one comes into action. This factor expresses itself in the blocking of the mitoses at anaphase, metaphase or prophase. In the first instance, these form atypical interphase nuclei (*e. g.*, dumb-bell- or ring-shaped nuclei) and later giant nuclei, which are in many cases polyploid. The so-called c-mitoses, in which the paralysis of the spindle is combined with a special kind of chromosome behaviour, give rise either to scattered micronuclei or to giant nuclei.

In the Landschütz tumour the cells outside the stem line show a slowing down of spindle formation. Owing to this the prophase (and possibly the interphase) becomes longer. Although their rate of division is slower than that of the stem line cells, they attain the majority, since the stem line continuously produces more of them. The growth process as a whole is slower than in the Ehrlich tumour. The cells which undergo endomitosis appear simultaneously with the lengthening of the prophase and are probably causally connected with this phenomenon. A proportion of the polyploid and giant cells arise through endomitosis. In an old tumour, finally, a number of mitoses are blocked and the spindles are paralysed for the same reasons and with the same results as in the Ehrlich tumour.

When considering the mechanism of the differentiation process, the most interesting question is why certain cells are pushed out of the stem line, and what this process implies. Since in an ascites tumour the environment is the same for all the cells, the course of growth and mitosis

which each cell takes must depend on its own genotype, *i. e.*, its gene composition. This, again, is determined by its chromosome complement. The mitotic mechanism of the stem line cells must be inherently unbalanced in a certain manner. Simultaneously with the production of »normal» cells which have the modal number of chromosomes, cells with a deviating chromosome make-up also arise. The variation in the behaviour of these deviating cells must obviously depend on their chromosomal composition, which varies in the different cells.

This line of thought necessitates the assumption that the stem line of the Ehrlich tumour has a tendency mainly to produce such deviating chromosome and gene combinations as accelerate spindle activity and favour the formation of multipolar mitoses, whereas in the Landschütz tumour combinations are produced which retard spindle activity and give rise to endomitoses. Whether or not these inherent differences depend on the fact that the Ehrlich tumour is tetraploid and the Landschütz tumour diploid, cannot be decided on the present evidence. To elucidate this point, it would be important to perform analyses corresponding to the present one on as many ascites strains as possible.

It has sometimes been claimed that it is not worth studying the abnormal mitoses and cells in neoplasms, since these, while adding little to the growth of the tumour, form the most easily disintegrating part of it. It is naturally true that the gross deviations from the normal cell type and mitotic type cannot form the basis of the bulk of the tumour growth. But if it can be shown to which processes in cell mechanics these abnormalities owe their origin, important information concerning the character of the tumour can be obtained, as is shown by the results of the present analysis.

Now the question arises whether such differences as are found between the Ehrlich and the Landschütz tumours can be established for solid tumours, especially for human neoplasms. As far as the human tumours have been investigated cytologically, they show neoplasia of the Ehrlich type. Features characteristic of the Ehrlich tumour (high value of the ratio M/P and great frequency of multipolar mitoses) occur in them in a pronounced degree, especially in far-advanced cases (TIMONEN and THERMAN, 1950; TIMONEN, 1950; THERMAN and TIMONEN, 1950, 1954). In extreme cases the ratio M/P may reach values over 20 and the frequency of multipolar mitoses values of about 50 %. The analogy between these and the Ehrlich ascites is thus indubitable. The only essential difference probably resides in the fact that an ascites tumour never can become old in the same sense as a solid tumour. As

far as the present author is aware, there is no example in the literature of a Landschütz-like solid human tumour, with a very low M/P ratio and abundant endomitoses. It is, however, not impossible that such a tumour type, if sought for, would be found.

Acknowledgements. — For the opportunity to carry out the present study at the Women's Clinic, the University of Helsinki, the author is greatly indebted to the Director of the Clinic, Professor AARNO TURUNEN, M. D.

For the ascites strains, as well as for advice in the cytological technique used, the author wishes to express his gratitude to Dr. ALBERT LEVAN, of Lund.

The present work has been supported financially by the Sigrid Jusélius' Stiftelse, President J. K. Paasikivi's Fund for Cancer Research, and the Damon Runyon Memorial Fund for Cancer Research.

SUMMARY

The mitotic mechanism of two mouse ascites tumours (the tetraploid Ehrlich tumour and the hyperdiploid Landschütz I tumour) has been investigated, with special reference to the relative frequencies (duration) of the mitotic phases and the occurrence of certain deviating mitotic types (multipolar divisions and endomitoses).

The samples for study were taken on the 3rd and the 7th day after inoculation (each sample from a different animal, total 24 mice) in order to analyse the changes occurring on aging of the tumour.

On the 3rd day both tumours consist mainly of cells dividing by normal mitosis; these are the so-called stem line cells, which are responsible for the growth of the tumour and maintain the continuity of the tumour from one host to another. In these cells the prophases and metaphases are about equally frequent, while the anaphases are much scarcer. This holds true for both the tumours.

On the 7th day the relative frequencies of the prophases and metaphases especially have undergone a considerable change. This change, however, has taken opposite directions in the two tumours. In the Ehrlich tumour the number of metaphases has greatly increased, the ratio M/P being 1,78, while in the Landschütz tumour the number of prophases has increased, the ratio M/P being 0,55. In both the tumours the percentage of anaphases has decreased.

In the light of the observations and scorings, this result is to be interpreted as follows: In the Ehrlich tumour the cells pushed out of the

stem line are characterized by a shortening of the prophase; in the Landschütz tumour, again, these are characterized by its lengthening. The former phenomenon implies precocity of the polarity, the latter the opposite state of affairs. In both tumours a number of mitoses are blocked on aging of the tumour. This happens especially at metaphase, but to a certain degree also at prophase, which leads to a decrease in the frequency of anaphases.

In the Ehrlich tumour multipolar mitoses are relatively frequent, depending on the abnormally rapid division and functioning of the centrosomes which are responsible for spindle formation. Endomitoses are practically absent.

In the Landschütz tumour multipolar mitoses are rare, while endomitoses are relatively abundant. The latter increase in number strongly on aging of the tumour. A clear positive correlation is to be found between the frequency of endomitoses and the duration of the prophase.

Literature cited

BAYREUTHER, K. 1952. Der Chromosomenbestand des Ehrlich-Ascites-Tumors der Maus. — Zschr. f. Naturforsch. 7 b: 554—557.

HIRONO, I. 1951. Some observations on the mitosis of the living malignant tumor cells. — Acta Pathologica Japonica 1: 40—47.

HSU, T. C. 1954. Cytological studies on HeLa, a strain of human cervical carcinoma. I. Observations on mitosis and chromosomes. — Texas Reports on Biology and Medicine 12: 833—846.

KLEIN, G. 1951. The production of ascites tumors in mice and their use in studies on some biological and chemical characteristics of neoplastic cells. — Thesis, Stockholm, 33 pp.

LEVAN, A. 1954. Colchicine-induced c-mitosis in two mouse ascites tumours. — Hereditas XL: 1—64.

LEVAN, A. and HAUSCHKA, T. S. 1952. Chromosome numbers of three mouse ascites tumours. — Hereditas XXXVIII: 251—255.

— and — 1953 a. Endomitotic reduplication mechanisms in ascites tumors of the mouse. — Journ. of the Nat. Cancer Inst. 14: 1—43.

— and — 1953 b. Nuclear fragmentation — a normal feature of the mitotic cycle of lymphosarcoma cells. — Hereditas XXXIX: 137—148.

MAKINO, S. 1951. The cycle of tumor cells in a transplant generation of the Yoshida sarcoma. — Proc. of the Japan. Acad. 27: 287—291.

— 1952 a. A cytological study of the Yoshida sarcoma, an ascites tumor of white rats. — Chromosoma 4: 649—674.

— 1952 b. Cytological studies on cancer. III. The characteristics and individuality of chromosomes in tumor cells of the Yoshida sarcoma which contribute to the growth of the tumor. — Gann 43: 17—37.

MAKINO, S. and HSU, T. C. 1954. Mammalian chromosomes *in vitro*. V. The somatic complement of the Norway rat, *Rattus norwegicus*. — Cytologia 19: 23—28.

MAKINO, S. and KANÔ, K. 1951. Cytological studies on cancer. II. Daily observations on the mitotic frequency and the variation of the chromosome number in tumor cells of the Yoshida sarcoma through a transplant generation. — Journ. Fac. Sc., Hokkaido Univ., Ser. VI, Zool. 10: 225—242.

— and — 1953. Cytological studies of tumors. IX. Characteristic chromosome individuality in tumor strain-cells in ascites tumors of rats. — Journ. of the Nat. Cancer Inst. 13: 1213—1234.

MAKINO, S. and YOSIDA, T. H. 1951. Cytological studies on cancer. I. Morphological and statistical observations on the abnormal mitosis in tumor cells of the Yoshida sarcoma through a transplant generation. — Journ. Fac. Sc., Hokkaido Univ., Ser. VI, Zool. 10: 209—224.

NAKAHARA, H. 1953. Cytological studies of tumors. XI. Observations of the multipolar division in tumor cells of ascites tumor of rats by phase microscopy. — Journ. Fac. Sc., Hokkaido Univ., Ser. VI, Zool. 11: 473—480.

OKSALA, T. 1954. Timing relationships in mitosis and meiosis. — Caryologia VI, Suppl.: 272—281.

THERMAN, E. and TIMONEN, S. 1950. Multipolar spindles in human cancer cells. — Hereditas XXXVI: 393—405.

— and — 1954. The prophase index and the occurrence of multipolar divisions in human cancer cells. — Hereditas XL: 313—324.

TIMONEN, S. 1950. Mitosis in normal endometrium and genital cancer. — Acta Obstetr. Gyn. Scand. 31, Suppl. 2: 1—88.

— 1956. The prophase index in the diagnosis of gynecological cancer. — Ann. Chir. Gyn. Fenn. (In the press.)

TIMONEN, S. and THERMAN, E. 1950. The changes in the mitotic mechanism of human cancer cells. — Cancer Research 10: 431—439.

TJIO, J. H. and LEVAN, A. 1954. Chromosome analysis of three hyperdiploid ascites tumours of the mouse. — Lunds Univ. Årsskr., N. F., Avd. 2, Bd 50, Nr 15: 1—29.

Contents

I. Introduction 161
II. Material and technique 163
III. Cytological description 163
1. Tetraploid Ehrlich tumour 163
2. Hyperdiploid Landschütz I tumour 166
IV. Statistical analysis 169
1. Methods 169
2. The Ehrlich tumour 171
3. The Landschütz I tumour 173
4. Comparison of the two tumours 175
V. Interpretation of the results 175
1. Normal mitoses 175
2. Endomitoses 178
3. Multipolar mitoses 180
VI. Stem line and the growth of the ascites tumour 181
Summary 186
Literature cited 187

ON ACCESSORY CHROMOSOMES IN FESTUCA PRATENSIS

III. FREQUENCY AND GEOGRAPHICAL DISTRIBUTION OF PLANTS WITH ACCESSORY CHROMOSOMES

By *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION

IN two previous papers (BOSEMARK, 1954 a and b) cytology and inheritance of accessory chromosomes in *Festuca pratensis* HUDS. (Meadow Fescue) have been described. The present paper deals with the frequency and geographical distribution of plants of this species with accessory chromosomes, found in natural populations in Sweden, and to a lesser extent in England.

Festuca pratensis is native throughout Europe, except in the most northern parts. Outside Europe it is indigenous to the western and inner parts of Asia. It has been introduced into North America.

In Sweden wild or naturalized populations of *Festuca pratensis* are fairly common in natural meadows and pastures, and on edges of ditches and road-sides, from the Province of Skåne in the south to the Province of Västerbotten in the north (HULTÉN, 1950). The species is widespread in the British Isles, but most common in England, where it is often abundant in water-meadows, in low-lying grassland, old pastures and on road-sides, especially on loamy and heavy soils (ARMSTRONG, 1950; HUBBARD, 1954).

The species thrives best on rich soils, preferably clays and marls, but thrives also on sandy soils, provided they are sufficiently moist. On rich, moist soils *Festuca pratensis* is a valuable grass for hay and grazing, whereas on poor soils, especially under dry conditions, its yield is poor and it cannot compete with more drought resistant grasses.

II. MATERIAL AND METHODS

In sampling Swedish populations whole plants were dug up at random and sent to the Institute of Genetics, Lund. Populations expected to

have originated more or less directly from cultivated material were avoided.

In England 30—40 ripe panicles were collected at each locality. In the following spring these were thrashed, and 50 seeds from each locality sown out in the green-house. At each locality notes were made about population size, soil type and humidity as well as influence of recent cultivation. All chromosome counts were made in root-tips fixed in chrome-acetic formalin, sectioned at 14 μ and stained in crystal violet. Further details on sample size, *etc.* will appear in the text.

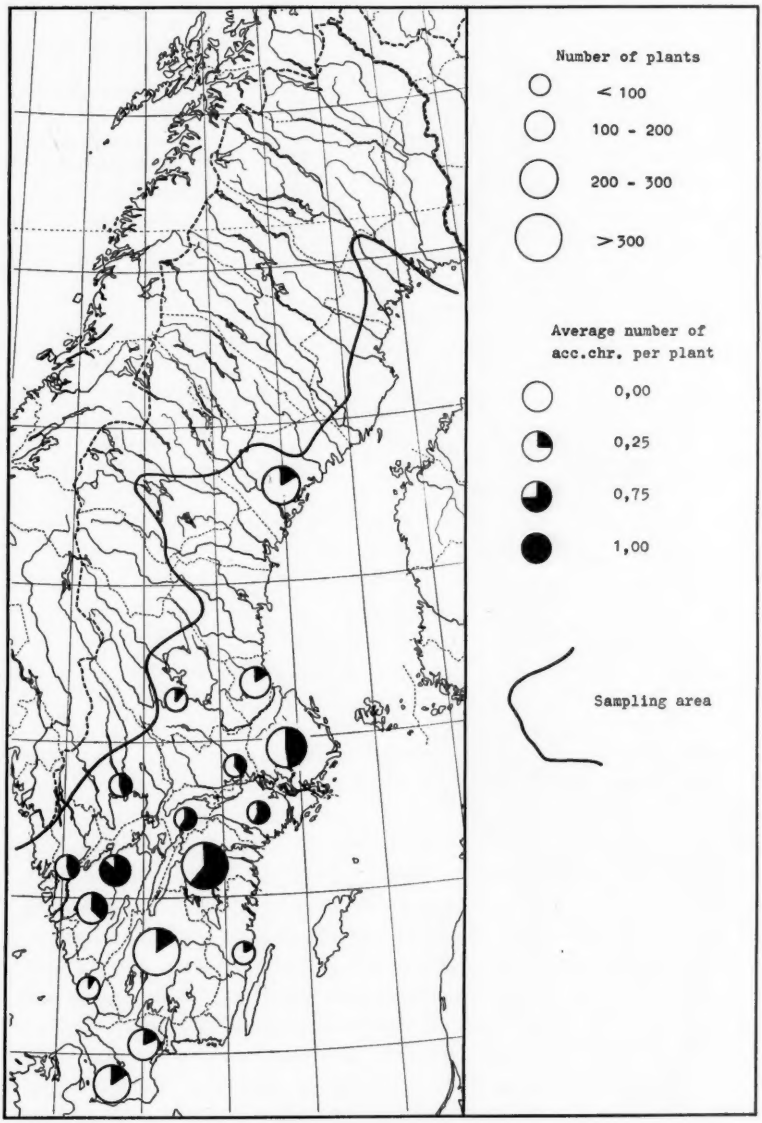
III. COLLECTIONS IN SWEDISH POPULATIONS

The first collections of *Festuca pratensis* from natural populations in Sweden were made in the summer of 1948, with the purpose of enlarging the material already put at my disposal by the Swedish Seed Association at Svalöf (BOSEMARK, 1950), and in this way to get not only more plants with accessory chromosomes for cytological studies and crossing experiments, but also some information on the frequency of such plants in wild populations in Southern and Central Sweden. The samples were very small, only 2—3 plants per locality. The resultant chromosome numbers are given in Table 1.

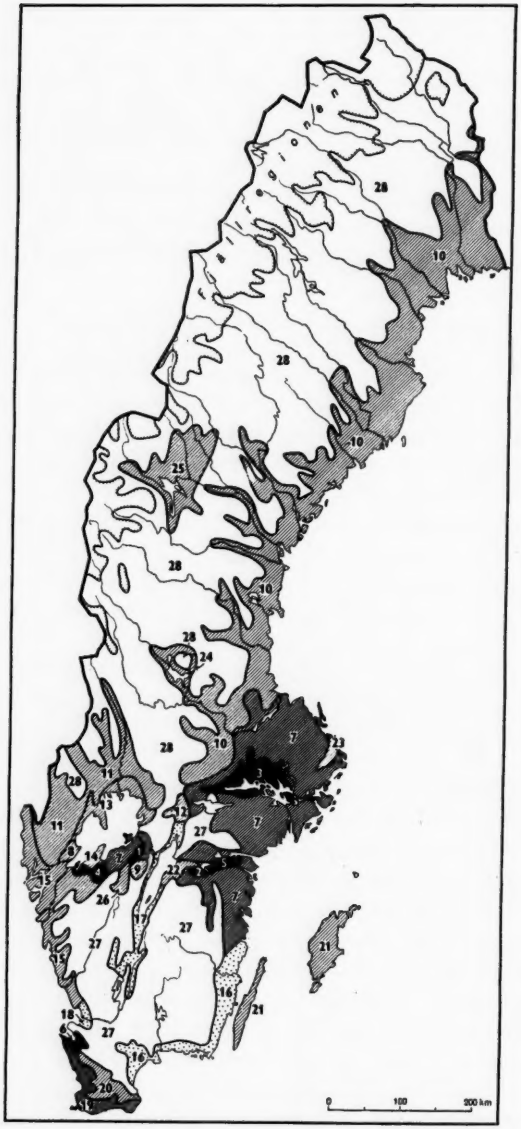
As seen, a relatively large portion of the plants had accessory chromosomes; the two lowest numbers dominating. In spite of the small sample size the data indicated that plants with accessory chromosomes are more frequent in the lowlands of Central Sweden than in surrounding areas.

To get more information about this a new collection was undertaken in 1949, covering more or less the whole distribution range of the species in Sweden (see Map 1). The average number of plants collected at each locality was about 5. These results are also included in Table 1.

The percentage of plants with accessory chromosomes as well as the average number of accessory chromosomes per plant is here slightly lower than for the material collected in 1948, but the distribution of the different chromosome numbers is the same. From this study it became clear that plants with accessory chromosomes exist throughout the distribution range of the species in Sweden, but that the frequency of such plants is different in different parts of the country. Similarly to the results in 1948 the highest frequencies were obtained in the lowlands of Central Sweden, especially in the three main regions of clay soil; the Lake Mälar-Hjälmar Plain, the Lake Väner Plain and the Östgöta Plain

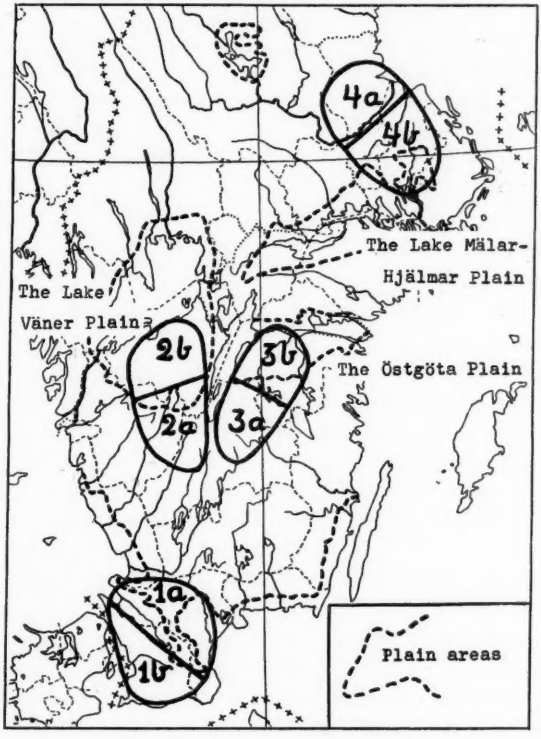


Map. 1. Frequency of accessory chromosomes in natural populations of *Festuca pratensis* in Sweden.



1—2. Heavy or very heavy clay
3—6. Heavy intermediate clay
7. Intermediate clay, fine mo-clay and heavy clay
8—9. Silty clay
10—11. Mo soils and silty clay
12. Fine mo-clay and intermediate clay
13—14. Fine mo-clay
15. Coarse mo-clay
16—18. Mo soils and sand
19—25. Moraine clay
26. Slightly clayey or clayey moraine
27—28. Archaean moraine

Map. 2. The division of Sweden into regions of cultivated soils according to surface-soil types.
(Reprinted from Atlas över Sverige.)



Map 3. The situation of the four districts on the boundaries between clayey and clayless soils mentioned in the text.

(see Maps 1 and 3). On the other hand the populations in the South Swedish Highlands and the Norrland Territory had a low frequency of plants with accessory chromosomes (see Map 1).

Attempts were now made to correlate this distribution with what was known about the ecological preferences of the species as well as the degree of its cultivation and population size, *etc.*, in different areas.

The interaction between the climatic conditions, the most important of which are temperature and humidity, and other natural conditions, primarily soil type and topography, is of greatest significance to the distribution of plants. As the climatic conditions in different parts of Southern and Central Sweden are fairly variable and showed no obvious correlation with the observed distribution of accessory chromosomes,

TABLE 1. *Frequency of accessory chromosomes in plants of Festuca pratensis collected in natural populations in Sweden in the years 1948 and 1949.*

Year	Number of plants with respective numbers of accessory chromosomes								Number of plants	Number of samples	Number of plants per sample	% plants with acc. chr.	M. acc. chr. per plant
	0	1	2	3	4	5	6	7					
1948	153	24	15	1	5	—	—	1	199	99	2,0	23,1	0,42
1949	701	73[1]	53	7[2]	6	3	3	1	847	186	4,6	17,2	0,31
Total	854	97	68	8	11	3	3	2	1046	285	3,7	18,4	0,33

[1] In two plants the accessory chromosomes were of the small type. (See BOSEMARK, 1954 a.)
[2] One plant had 2 standard+1 small accessory chromosome.

TABLE 2. *Correlation between the clay content of the soil and the frequency of plants with accessory chromosomes in Sweden.*

Soil type and area	Number of plants with respective numbers of accessory chromosomes								Number of plants	% plants with acc. chr.	M. acc. chr. per plant
	0	1	2	3	4	5	6	7			
A. Heavy clay soils:											
1. Southern and Central Sweden	117	25	21	3	5	—	—	2	173	32,4	0,64
B. Light clay soils:											
1. Southern and Central Sweden	141	25	19	2	4	2	2	—	195	27,7	0,55
2. The Norrland Territory	143	11	10	1	—	—	—	—	165	13,3	0,21
C. Slightly clayey or clay-free soils:											
1. Southern and Central Sweden	268	28	13	—	1	1	1	—	312	14,1	0,22
2. The Norrland Territory	185	8	5	2	1	—	—	—	201	8,0	0,14
Total	854	97	68	8	11	3	3	2	1046	18,4	0,33

χ^2-analysis of the data from Table 2.

Soil type and area	Number of acc. chr. 0	1	2–7	Total
A1	117	25	31	173
B1	141	25	29	195
B2	143	11	11	165
C1	268	28	16	312
C2	185	8	8	201

A1/B1	$\chi^2 = 0{,}987$	$0{,}7 > p > 0{,}5$
A1/C1	$\chi^2 = 26{,}522$	$p < 0{,}001$
B1/C1	$\chi^2 = 17{,}280$	$p < 0{,}001$
B2/C2	$\chi^2 = 2{,}936$	$0{,}3 > p > 0{,}2$

the interest was centered on studying the relationship between frequency of accessory chromosomes and edaphic and biotic factors, especially soil type, in different areas. As seen from the map (Map 2) of Sweden divided into regions of cultivated soils according to surface-soil types, the soils in the lowlands of Central Sweden are more or less heavy clays; whereas the South Swedish Highlands, and most other parts of Sweden, except south-west of Skåne, consist of only slightly clayey or clayless moraine and sand. Most of the clays in the lowlands of Central Sweden, except smaller parts of the Lake Väner Plain and the Östgöta Plain, are sedimentary clays from late quaternary time. The clays in south-west Skåne, and the two smaller areas just mentioned, are mainly of the moraine clay type. At this stage of the investigation, however, very few plants had been collected in Skåne, due to the difficulties in getting authentic wild material from this highly cultivated area.

As the clay content of a soil greatly affects its physical as well as chemical properties, one often bases classification of mineral soils upon clay content. If the humus or mould content of a soil is satisfactory, the clay contents are a good measure of the agricultural value of the soil. Thus taking into account the preferences of *Festuca pratensis* with regard to the soil, the frequency of accessory chromosomes seems to be highest in those areas which have the most suitable edaphic conditions for the species.

To illustrate this the material from 1948 and 1949 has been arranged according to the clay contents of the soil (Table 2). The grouping of the material under three headings, heavy clay soils, light clay soils and slightly clayey or clay-free soils, was based on notes made in the field and a map over the distribution of the different soils of the arable land, similar to Map 2 in this paper, but far more detailed.

From the table and the statistical analysis it is evident that in Southern and Central Sweden plants that grow on more or less clayey soils more often have accessory chromosomes than plants growing on clayless moraine and sand. The separation of the Norrland Territory from the rest of Sweden has been done mainly to show that the material collected in this area does not specially impress the figures for the clayless soils, although such a separation is justifiable also for other reasons. Besides differences in edaphic and especially climatic conditions, the species is much less frequent in Norrland than in the rest of Sweden and also more closely bound to cultivation. Though there is the same tendency in the material from Norrland with respect to the distribution of the accessory chromosomes, the lower average frequency in Norrland might thus have its special causes.

To get still better evidence for the existence of such a correlation between frequency of accessory chromosomes, and the clay content of the soil, collections were made in 1951 and 1952 in populations from four different districts in Sweden, so chosen that within each district the boundaries between clay soil areas and clayless areas were covered, as well as areas on each side of these boundaries. The number of plants collected in each population was about 20. The four different districts have been marked out on Map 3. The result of the analysis of the material which consisted of 1560 plants from 79 localities, is reported in Table 3. To give an idea of the distribution of the chromosome numbers in the individual populations in a district, the original data for one of the districts are given in Table 4. The statistical analysis of the results, summarized in Table 3, is done on the basis of the mean values for the individual populations.

The result of this investigation agrees quite well with what was expected on the basis of the earlier collections, except for area 1 b in Skåne. For the other districts the difference between clayey and clayless areas with respect to percentage of plants with accessory chromosomes as well as average number of accessory chromosomes per plant is significant to a higher or lower degree. The absence of such a difference in district 1 in Skåne does not necessarily mean that the relationship between soil type and frequency of accessory chromosomes is only accidental, or that there is no direct relationship between these two factors. For, unfortunately, quite a number of the samples from the clay soil area in Skåne are not very representative for the area. This is due to the fact that in this highly cultivated area, with little waste land, it is difficult to find wild or naturalized populations growing on the better

TABLE 3. *The frequency of plants with different numbers of accessory chromosomes in the four districts on the boundaries between clayless and clayey soils.*

(a=clayless soils, b=clayey soils.)

District	Number of plants with respective numbers of accessory chromosomes						Total number of plants	% plants with acc. chr.	M. acc. chr. per plant
	0	1	2	3	4	5			
1 a	86	12	3	—	—	—	101	14,9	0,18
1 b	287	28	16	1	—	—	332	13,6	0,19
2 a	188	25	16	1	—	—	230	18,3	0,26
2 b	71	25	12	6	6	3	123	42,3	0,86
3 a	167	14	8	—	—	—	189	11,6	0,16
3 b	150	34	23	8	8	3	226	33,6	0,67
4 a	127	14	7	—	—	—	148	14,2	0,19
4 b	144	32	26	4	3	2	211	31,8	0,56
Total	1220	184	111	20	17	8	1560	21,8	0,37

Statistical analysis of the data from Table 3 based on the mean values for the individual populations.

District	Between area a and b			Within district			Total		Quotient	p-value
	Degrees of freedom	Sum of squares	Mean square A	Degrees of freedom	Sum of squares	Mean square B	Degrees of freedom	Sum of squares		
1	1	0,0019	0,0019	20	0,4120	0,0206	21	0,4139	B/A = 10,84	insignificant
2	1	1,4366	1,4366	17	1,1581	0,0681	18	2,5947	A/B = 21,10	< 0,001
3	1	1,1858	1,1858	17	2,0870	0,1228	18	3,2728	A/B = 9,66	0,01—0,001
4	1	0,5889	0,5889	17	2,3185	0,1364	18	2,9074	A/B = 4,32	(0,2)—0,05

soils representative for the real plain area. If one tries to avoid cultivated material, one is no longer collecting on the typical clay soil.

As already mentioned, there are other factors than soil type which might be, at least in part, responsible for the distribution found. Of these the following two are probably of importance: (1) The abundance, size and longevity of wild and naturalized populations. (2) The extent of cultivation of the species and the frequency of plants with accessory chromosomes in the commercial strains used.

(1) Although *Festuca pratensis* is spread over a large part of Sweden, the species is certainly more common in the lowlands. As already mentioned, in the Norrland Territory and in the South Swedish Highlands, large wild or naturalized populations are seldom met with; most

TABLE 4. *The distribution of different chromosome numbers in the clayless and clayey areas of district number 3.*

Area	Sample	Number of plants with respective numbers of accessory chromosomes						Total number of plants	M. acc. chr. per plant
		0	1	2	3	4	5		
a	11/52	18	2	1	—	—	—	21	0,19
a	12/52	22	1	1	—	—	—	24	0,13
a	13/52	20	2	2	—	—	—	24	0,25
a	14/52	17	1	—	—	—	—	18	0,06
a	15/52	11	—	2	—	—	—	13	0,31
a	16/52	20	2	—	—	—	—	22	0,09
a	17/52	18	2	1	—	—	—	21	0,19
a	18/52	19	1	1	—	—	—	21	0,14
a	19/52	22	3	—	—	—	—	25	0,12
Total area a		167	14	8	—	—	—	189	0,16
b	1/52	13	5	3	2	—	—	23	0,74
b	2/52	16	5	2	1	—	—	24	0,50
b	3/52	17	2	—	1	2	—	22	0,59
b	4/52	5	5	6	2	3	2	23	1,96
b	5/52	16	3	2	—	—	—	21	0,33
b	6/52	12	6	4	1	—	—	23	0,74
b	7/52	15	1	2	—	1	—	19	0,47
b	8/52	20	—	2	—	1	—	23	0,35
b	9/52	20	1	1	1	1	—	24	0,42
b	10/52	16	6	1	—	—	1	24	0,54
Total area b		150	34	23	8	8	3	226	0,67

being small, shortlived and isolated. This not only influences the possibilities of the accessory chromosomes spreading from one population to another, but also limits the effect of the cytological mechanism for their numerical increase which has been demonstrated to be at work in *Festuca pratensis* (BOSEMARK, 1954 a and b). Also the result of natural selection is likely to be influenced by population size and density.

(2) *Festuca pratensis* can be considered native to Sweden, but in spite of this, and its value as a herbage grass, it has not been cultivated here for a very long time. Not until the beginning of the twentieth century was the cultivation of any importance, and then occurred mainly in Skåne, probably through influence from Denmark, where the use of Meadow Fescue in leys and pastures was more widespread. Thus most of the material cultivated in Southern Sweden was of Danish origin, and of little value for Central and Northern Sweden. With the appearance of strains which shoot and flower later the cultivation of *Festuca pra-*

TABLE 5. *Frequency of plants with accessory chromosomes in Swedish commercial strains of Festuca pratensis.*

Strain	Number of plants with respective numbers of accessory chromosomes					Total number of plants	% plants with. acc. chr.	M. acc. chr. per plant
	0	1	2	3	4			
Svalöf's Early	80	15	14	2	1	112	29	0,47
Svalöf's Medium	113	6	5	—	—	124	9	0,13
Svalöf's Late	129	8	2	—	—	139	7	0,09
Svalöf's Bottnia I	109	5	2	—	—	116	6	0,08
Weibulls Mimer	119	14	4	2	—	139	14	0,20
Hammenhög's Fepra	53	6	1	—	—	60	12	0,13

tensis in these parts of Sweden has become more important (JULÉN and HALLING, 1953). Still the cultivation is, however, more important in Skåne than in any other part of Sweden. NILSSON-LEISSNER *et al.* (1950) have estimated, on the basis of hay inventations, that Meadow Fescue together with other grasses and clover is used in about 50 per cent of the leys in Southern Skåne, constituting about 5 per cent of the seeds in the mixtures. For all Sweden about 10 per cent of the leys contain Meadow Fescue (JULÉN and HALLING, 1953).

To get some idea how much the cultivation of the species, and the constitution of the different commercial strains could have influenced the distribution of accessory chromosomes, samples from six Swedish strains of *Festuca pratensis* were investigated for their chromosome constitution (Table 5). The strains have all been on the market for several years; Svalöf's Late since 1917.

As seen, accessory chromosomes are present in all the strains, although the frequency is different. This is not surprising, since besides intercrossing with wild material during seed multplication, at least some of the strains are derived from wild Swedish material. If the spontaneous material has accessory chromosomes, and a low number does not markedly affect vitality, it is likely that one should also find them in the cultivated strains.

The area of arable land in Sweden coincides very well with the distribution of the different soils in such a way, that the clay soil areas in the plains are to a high degree agricultural land, while in the rest of Sweden the area of forests dominates. Thus there is a correlation between the degree of cultivation in general, as well as between the area of leys for hay and grazing, measured in absolute figures, on the one hand, and the frequency of accessory chromosomes on the other. If the area of leys is measured in per cent of arable land, the previously mentioned plains, however, have far from the highest figures.

As *Festuca pratensis* is more widespread as a herbage grass in Skåne than in any other province, and as it is not commonly cultivated in many of the areas with a high frequency of accessory chromosomes, it is not very likely that the cultivated material is responsible for the high frequency of accessory chromosomes in the lowlands of Central Sweden; whereas it cannot be excluded that it has influenced the situation in south-western Skåne. One should in this connection also remember that where seed production does not occur, the leys are generally cut or grazed well before *Festuca pratensis* starts flowering.

IV. COLLECTIONS IN ENGLISH POPULATIONS

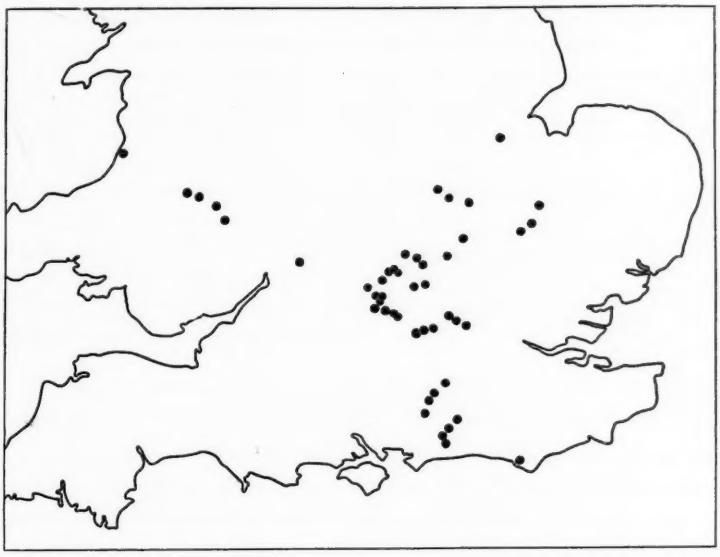
In 1951 seeds of *Festuca pratensis* were collected in pastures and water-meadows in Southern and Central England. According to English botanists, the species is considered native here, and the climatic as well as edaphic conditions especially in the water-meadows are more or less optimal (HUBBARD, personal communication).

As the water-meadows are often flooded in the winter, they are very seldom sown or cultivated. Many farmers stated that plowing and cultivation had not taken place for as long as they could remember, thus some of the meadows have probably been left relatively undisturbed for more than 100 years. Cultivated material should therefore not interfere with the results. As the meadows and pastures are grazed from early spring till late in the autumn they are, however, not to be considered natural. The grazing as well as the trampling of the cattle may also cause a special selection in the plant material growing at such localities.

In spite of this, it was considered that the study would give valuable information, and might also help to clarify the situation in Sweden. Similarly to the situation here, a fairly high frequency of accessory chromosomes was expected in the English populations. However, the result was quite the opposite (Table 6).

The chromosome numbers were determined in altogether 1654 plants from 47 localities (see Map 4) with about 35 plants per locality. Out of these 47 localities only 11 had plants with accessory chromosomes, and not more than 4 had frequencies comparable with those commonly found in Central Sweden (see Table 4). In Tables 7 and 8 a comparison has been made between the materials from Sweden and England.

Whereas in the majority of the populations in England accessory chromosomes are lacking or only present in such low frequency that



Map 4. The situation of the localities in England and Wales.

they have not appeared in the samples, most Swedish populations have plants with accessory chromosomes to a varying amount. In the English material no marked correlation was found between frequency of accessory chromosomes and population size, soil type, humidity or influence of cultivation, but this may very well depend on the small variation between the localities in most of these respects. All populations, however, which for one reason or other could be classified as very old, completely lacked accessory chromosomes, whereas the few populations with fairly high frequencies were all young. Whether this is the result of a selection against accessory chromosomes in the old populations, or if the original material invading these localities lacked accessories is not known.

From Table 5 it is known that in Sweden, where most of the natural populations have accessory chromosomes to a varying degree, accessories are present also in all examined commercial strains. Two English strains obtained from the Welsh Plant Breeding Station, were studied for their chromosome constitution. From these strains, namely LD49 and KD49, 59 and 63 individuals, respectively, were studied, but all the plants had fourteen normal chromosomes. Although this is a very

TABLE 6. *Chromosome numbers in natural populations of Festuca pratensis in England.*

Number of plants with respective numbers of accessory chromosomes					Total number of plants	Number of samples	% plants with. acc. chr.	M. acc. chr. per plant
0	1	2	3	4				
1582	42	23	6	1	1654	47	4,4	0,07

TABLE 7. *Comparison between the frequency of accessory chromosomes in Swedish and English populations of Festuca pratensis.*

	Number of plants with respective numbers of accessory chromosomes								Total number of plants	% plants with. acc. chr.	M. acc. chr. per plant
	0	1	2	3	4	5	6	7			
Sweden	2074	281	179	28	28	11	3	2	2606	20,4	0,35
England	1582	42	23	6	1	—	—	—	1654	4,4	0,07

TABLE 8. *Comparison between population samples of comparable size from Sweden and England with respect to presence and absence of plants with accessory chromosomes.*

	Number of samples		Sample size	% samples with. acc. chr.
	With acc. chr.	Without acc. chr.		
Sweden	59	18	20	77
England	11	37	35	23

$\chi^2 = 34{,}613$; $p < 0{,}001$

meagre material on which to base any conclusions, there is a parallel between the wild and cultivated materials in the two countries, which may be of some significance.

V. CULTIVATED AND WILD MATERIAL FROM OTHER COUNTRIES

Out of thirteen commercial strains from Holland, Denmark, Finland, Germany, and Canada, only four, *viz.* one Danish and three German had accessory chromosomes.

The Plant Breeding Station at Wageningen has kindly supplied me with a sample of seeds, collected after open pollination of an artificial population constituted by wild plants collected all over Holland. The distribution of the different chromosome numbers in a sample from this seed-lot is reported in Table 9.

A few seed samples of wild material of *Festuca pratensis* from Poland, Germany and Italy have revealed the existence of the same type of accessory chromosomes as found in Sweden and England. It is also

TABLE 9. *Chromosome numbers in progeny plants from an open-pollinated artificial population from Holland.*

Number of plants with respective numbers of accessory chromosomes			Total number of plants	% plants with acc. chr.	M. acc. chr. per plant
0	1	2			
102	3	3	108	5,6	0,08

known that they exist in Finnish populations (RANCKEN, 1934). It is thus likely that accessory chromosomes are present in populations of *Festuca pratensis* over most of its distribution range in Europe, although the frequency is probably different.

VI. DISCUSSION

To give a correct interpretation of the data now presented is difficult. If we to begin with restrict ourselves to the situation in Sweden, our present knowledge can be summarized as follows:

(1) Accessory chromosomes are present all over the distribution range of the species in Sweden. The frequencies in different areas, however, are markedly different.

(2) Accessory chromosomes are most frequent in the lowlands of Central Sweden, and these areas are also favourable to the species from an ecological point of view.

(3) There is a marked positive correlation between the clay content of the soil and the frequency of plants with accessory chromosomes, except in Skåne.

(4) In the lowlands of Central Sweden the species is more common and the populations larger and more persistent than in, for instance, the South Swedish Highlands. This influences the spreading of the accessory chromosomes and probably also the result of natural selection in the different areas.

(5) All Swedish commercial strains studied have accessory chromosomes to a varying degree.

(6) From the distribution of the different chromosome numbers in the collected material and the results from earlier crossing experiments (BOSEMARK, 1954 b), it is quite clear that there is an effective mechanism at work limiting the number of accessory chromosomes in natural populations. A negative effect of high numbers of accessory chromosomes on plant weight and fertility has also been experimentally proved

(BOSEMARK, unpublished). On the other hand I have not been able to find any deleterious effects of low numbers of accessories, *viz.* one and two, which are the most common in natural populations. Instead, there are slight indications that, under certain conditions, low numbers of accessory chromosomes might have a positive effect on plant weight (BOSEMARK, unpublished).

(7) Cytological studies and crossing experiments (BOSEMARK, 1954 a and b) have shown that the behaviour of the accessory chromosomes is at least to some extent dependent on environmental conditions, including the genotype of the host plant. This might influence the result of natural selection under different climatic and edaphic conditions.

On the basis of these facts the following explanation of the differences in frequency of plants with accessory chromosomes in different parts of Sweden is given.

The high frequency of accessory chromosomes among the populations in the lowlands of Central Sweden should depend on lack of selection against, or even selection for, plants having a low number of accessory chromosomes in combination with an effective non-disjunction mechanism and a low degree of meiotic elimination. The species being more common and the populations larger and more permanent also help to spread and maintain the accessories in these areas. The cultivated material may help to spread the accessory chromosomes to populations where they have not been before, but it is unlikely that it is responsible for the high frequencies.

The low frequency in the South Swedish Highlands and most parts of the Norrland Territory, on the other hand, should depend on a combined action of a number of factors, out of which the following are probably the most important:

(a) The populations are smaller, isolated and more short-lived, which makes the spreading of the accessories more difficult.

(b) The ecologic conditions are less favourable, which might render even low numbers of accessory chromosomes deleterious, or at least make a possible positive selection for accessories ineffective.

(c) The non-disjunction mechanism may very well be less effective, and the meiotic elimination of accessories higher under unfavourable conditions. Concerning the low frequencies met with in Skåne, I have already tried to give an explanation.

There is, however, also the possibility that populations growing on the richer soils react more favourably to accessory chromosomes than populations in areas less suited to the species as a result of genotypic

differences. Although there is no experimental evidence for such differences, one can imagine how they could have arisen. As *Festuca pratensis* belongs to the group of plants which have probably invaded Sweden over both the Baltic countries and Denmark (HULTÉN, 1950), it is possible that different areas received material differing with respect to the average genotypic constitution, as well as frequency of accessory chromosomes. Such initial differences, if they have ever existed, could have played a rôle in the development of a continuous or clinal variation as a result of differences in the ecologic conditions prevailing in different areas. That the situation to-day should directly reflect such possible initial differences is unlikely, since the gene exchange between the different areas is probably fairly free. It is also possible that if the accessory chromosomes, as well as the species itself, have been present for a longer time and more abundant in the lowlands, an adaptation to accessory chromosomes has developed further here than in the other areas.

Such an adaptation to accessory chromosomes has been discussed already by ÖSTERGREN (1947). Based on the hypothesis that accessory chromosomes can be considered parasitic, ÖSTERGREN made a diagram intended to show a possible effect on plant vigour of adaptation to accessory chromosomes. Although, after some degree of adaptation, plants with low numbers of accessory chromosomes are more vigorous than plants without, they are all inferior to such plants before the adaptation process took place. On the basis of his diagram, ÖSTERGREN points out that it would be a mistake to conclude that in this case the accessory chromosomes have improved the population by their presence.

Although it is not the purpose of this paper to prove a positive selective value of the accessory chromosomes in *Festuca pratensis*, it should be pointed out that ÖSTERGREN's diagram considers only one possibility which is closely associated with the idea of the parasitic nature of accessory chromosomes. Parallel to mutant genes it is, however, not unlikely that accessory chromosomes can decrease viability together with one genotype or in one environment, whereas when placed on another genetic background or in another environment viability is instead increased. Under such circumstances the shape of the viability curves would change correspondingly. Recently, DOBZHANSKY and SPASSKY (1954) have demonstrated in *Drosophila pseudoobscura* how extremely sensitive certain karyotypes are to environmental changes. A variant which is deleterious under some conditions can very well become useful under conditions only slightly different.

With respect to the British populations it is still more difficult to choose between different possible explanations. If a low number of accessory chromosomes under the conditions prevailing here have no effect at all, or a slightly negative effect, they could have been lost in the old populations with their hard competition. This then shows that the non-disjunction mechanism alone is at least not always sufficient to balance the negative effect. Judging from the Swedish populations, where in general a low frequency of accessory chromosomes is found also at localities where the environmental conditions are less favourable, it is perhaps more likely that accessory chromosomes were not present, or at least very infrequent, in the material that originally invaded these English localities. That accessories are still infrequent or not present in the old pastures and meadows could depend on difficulties for material coming from outside these localities to compete successfully with the well adapted material already present. The diffference between Sweden and England with respect to the over-all frequency of accessory chromosomes might thus very well depend on historical factors.

Although accessory chromosomes have been described in a fairly large number of species of plants as well as animals, more detailed studies of the frequency and distribution in natural populations have been made only for a few of them. WHITE (1951 a and b) describes the frequency and distribution of supernumerary chromosomes in natural populations, among other species, the grasshopper *Trimerotropis sparsa*; LEWIS (1951) has done the same in *Clarkia elegans*; and SPARROW *et al.* (1952) in *Trillium erectum*. These authors all find, though they are working with quite different materials, that there is a geographical distribution of these chromosome elements in such a way that some populations have accessories, others lack them. Within those populations which have accessory chromosomes, these are present only in a smaller or larger portion of the individuals constituting the population. On the basis of their observations the authors discuss the origin of the accessories, and the possibility that they have a positive selective value. WHITE arrives at the conclusion that in the *Trimerotropis* one accessory chromosome under most conditions slightly increases viability or fertility, whereas two or more decrease fitness to some extent. These conclusions are based on the fact that he has not been able to find any mechanism for numerical increase of the accessory chromosomes during the spermatogenesis. Lacking such a mechanism also on the female side and with the accessory chromosomes entirely neutral or negative, they should soon be eliminated from natural populations, whereas a

positive effect regardless of number of accessory chromosomes present, should lead to all individuals in a population carrying a varying number of accessory chromosomes. WHITE (1951) also points out that the genetic property of accessory chromosomes cannot be considered apart from the genotypic constitution of the individuals and populations in which they occur, as well as the environment inhabited by these populations.

LEWIS means that the accessory chromosomes in *Clarkia* may have a hidden physiological effect which is very difficult to measure, but nevertheless can be of importance under special conditions.

Besides the wild materials now mentioned, the extensive studies of accessory chromosomes in *Zea*, *Secale* and *Sorghum* have shown that the frequency of accessory chromosomes varies highly from one strain to another.

In most commercial strains of dent, pop and flour corn B-chromosomes are rarely found, whereas in some strains of sweet corn they are quite common (RANDOLPH, 1941). In a study of maize from Indian tribes in North America, LONGLEY (1938) found B-chromosomes in 14 of 33 varieties. He also found a negative correlation between number of knobs on the normal chromosomes and number of B-chromosomes. As the average number of knobs varies between different areas, it is likely that there is a geographical distribution also of the B-chromosomes.

MÜNTZING (1954) has demonstrated that the standard type of accessory chromosome in rye is present in populations from widely different parts of the world. The frequency is, however, very different. In commercial varieties in Europe accessory chromosomes are rare or absent, whereas in primitive strains from Asia they are sometimes very common. Thus accessory chromosomes were found in all seven strains obtained from Anatolia in Turkey, in one out of five strains from Afghanistan and in one from Transbajkal. In the latter strain 28 per cent of the plants had accessory chromosomes. Finally, in a rye variety from Chorea accessory chromosomes seem to be present in practically every individual (OINUMA, 1952; MÜNTZING, 1954).

The more fragmentary data from *Sorghum* (GARBER, 1950) indicate a similar situation.

Although the great differences between different cultivated strains of maize and rye, with respect to the frequency of accessory chromosomes, are probably largely dependent on the intensity of the selection for high fertility and productivity to which they have been subjected, LONGLEY's data in maize and MÜNTZING's in rye indicate that also in these species,

at least in primitive varieties, environmental conditions and genotypic constitution of different strains are of significance.

Recently, FRÖST (in MÜNTZING, 1954 and FRÖST, unpublished) has found a very marked geographical distribution of plants with accessory chromosomes in *Centaurea scabiosa*. FRÖST finds that in populations with accessory chromosomes the frequency varies, with the highest frequencies occurring in those areas which he classifies as most natural for the species, while the lower frequencies are found in less suitable areas, if accessory chromosomes are present at all. As extensive crossing experiments have revealed no signs of a mechanism for numerical increase of the accessories, but instead a slight but consistent meiotic elimination, FRÖST's data strongly suggest that low numbers of accessory chromosomes under special circumstances have a positive selective value in this species. A positive effect of low numbers of accessory chromosomes on the vegetative development has also lately been experimentally proved in *Centaurea* (FRÖST, 1954).

Although it is not justifiable to draw any close parallel between *Festuca* and *Centaurea*, one species having a mechanism for numerical increase, the other obviously lacking such a mechanism, it is in my opinion quite possible that a similar process underlays the geographical distribution of plants with accessory chromosomes in both species.

SUMMARY

(1) The present paper deals with the frequency and geographical distribution of plants with accessory chromosomes in *Festuca pratensis* HUDS., found in natural populations in Sweden, and to a lesser extent in England.

(2) Accessory chromosomes were found to be present all over the distribution range of the species in Sweden, but the frequency is different in different areas.

(3) There is a marked correlation between frequency of accessory chromosomes and the clay content of the soil in Sweden, except in the Province of Skåne. This deviation is considered to depend on influence of cultivation in general as well as of the species in question.

(4) All Swedish commercial strains studied have accessory chromosomes to a varying degree.

(5) The different frequencies in different parts of Sweden and the positive correlation between clay content of the soil and frequency of accessory chromosomes are explained mainly on the basis of the ecolog-

ical preferences of the species. A number of other factors which might be of importance are also considered.

(6) In the material collected in England the over-all frequency of accessory chromosomes is much lower, and no marked correlation between ecological factors and frequency of accessory chromosomes was found.

(7) All old and well established populations in England were found to lack accessory chromosomes or have them in such a low frequency that they did not appear in the samples. Of the few populations which had accessory chromosomes the highest frequencies were obtained in the youngest ones.

(8) It is suggested that the material that originally invaded the old meadows and pastures in England was relatively free from accessory chromosomes, and that more recent introduction of plants with accessory chromosomes has been limited by hard competition in combination with the high degree of adaptation of the material already present.

Literature cited

ARMSTRONG, S. F. 1950. British grasses and their employment in agriculture; ed. 3. — Cambridge.

BOSEMARK, N. O. 1950. Accessory chromosomes in *Festuca pratensis* HUDS. — Hereditas XXXVI: 366—368.

— 1954 a. On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. — Hereditas XL: 346—376.

— 1954 b. On accessory chromosomes in *Festuca pratensis*. II. Inheritance of the standard type of accessory chromosomes. — Hereditas XL: 425—437.

DOBZHANSKY, TH. and SPASSKY, N. 1954. Environmental modification of heterosis in *Drosophila pseudoobscura*. — Proc. Nat. Acad. Sci. 40: 407—415.

FRÖST, S. 1954. The genetic effect of accessory chromosomes in *Centaurea scabiosa*. — Hereditas XL: 529—533.

GARBER, E. D. 1950. Cytotaxonomic studies in the genus *Sorghum*. — Univ. Calif. Publ. Bot. 23: 283—362.

HUBBARD, C. E. 1954. Grasses. — Penguin Books.

HULTÉN, E. 1950. Atlas över växternas utbredning i Norden. — Stockholm.

JULÉN, G. and HALLING, S. 1953. Resultat från försök med svenska ängssvingelstammar. (Results from trials with Swedish strains of meadow fescue.) — Sv. Utsädesf. Tidskrift 63: 181—200.

LEWIS, H. 1951. The origin of supernumerary chromosomes in natural populations of *Clarkia elegans*. — Evolution V: 142—157.

LONGLEY, A. E. 1938. Chromosomes of maize from North American Indians. — Jour. Agr. Research 56: 177—195.

MÜNTZING, A. 1954. Cyto-genetics of accessory chromosomes (B-chromosomes). — Caryologia, Suppl. Vol. 282—301.

NILSSON-LEISSNER, G., WEIBULL, W., and NORDÉN, F. 1950. Fröodling av lantbruksväxter. — Stockholm.

OINUMA, T. 1952. Karyomorphology of cereals. — The Biolog. J. of the Okayama Univ. 1: 12—71.

RANCKEN, G. 1934. Zytologische Untersuchungen an einigen wirtschaftlich wertvollen Wiesengräsern. — Acta Agralia Fenn. 29: 1—92.

RANDOLPH, L. F. 1941. Genetic characteristics of the B chromosomes in maize. — Genetics 26: 608—631.

SPARROW, A. H., POND, V., and SPARROW, R. C. 1952. Distribution and behaviour of supernumerary chromosomes during microsporogenesis in a population of *Trillium erectum* L. — The Am. Nat. LXXXVI (830): 277—292.

WHITE, M. J. D. 1951. A cytological survey of wild populations of *Trimerotropis* and *Circotettix* (Orthoptera, Acrididae). II. Racial differentiation in *T. sparsa*. — Genetics 36: 31—53.

— 1951. Cytogenetics of Orthopteroid insects. — Advances in Genetics 4: 267—330.

ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas XXXIII: 261—296.

ON THE DIFFERENTIAL ACTION OF MUTAGENIC AGENTS

By *NILS NYBOM*

BALSGÅRD FRUIT BREEDING INSTITUTE, FJÄLKESTAD, SWEDEN

THE question whether different mutagenic agents bring about identical genetic effects or not has been up for discussion several times. The common answer, and the one usually found in text books, is that the mutations originate »at random» and that they are the same whether spontaneous or induced by various means.

But there have also been indications of differences between induced and spontaneous mutations. Also, recent evidence points to rather great divergences in action both between various chemical mutagens and between chemicals and radiations. This has been recorded either at the cytological level (*cf., e. g.,* GRAY, 1954) or as qualitative differences in mutation yield (*e. g.*, KÖLMARK, 1953 and MACKEY, 1954).

The non-ionizing radiations, *i. e.*, in the first place ultraviolet light, have long been known to deviate from the ionizing ones in cytological effects. Moreover, some authors held the opinion that only ultraviolet light would be able to induce positive (progressive) genetical changes, whereas »X-rays do not give rise to true gene mutations but only to destructive changes» (LEFEVRE *et al.*, 1953). These ideas are difficult to reconcile with the fact that undisputable progressive mutations have been induced by x-rays in cultivated plants (*cf.*, *e. g.*, GUSTAFSSON and TEDIN, 1954) and also with the author's own studies on the viability of translocation homozygotes in barley to be described below. GILES *et al.* (1955) have also found that »x-rays can induce dominant, positive mutations resulting in the restoration of specific biochemical syntheses» in *Neurospora.*

The ionizing radiations may suitably be grouped and treated according to the linear ionization density they bring about along the track of the ionizing particle in the tissue; γ-rays with *ca.* 8 such ionizations per μ particle track, x-rays usually with some 100, neutrons of various energies with 500—2000 and, finally, α-particles and fission fragments from about 2000 to several 10000 ionizations per μ.

Comparisons between the genetical action of these various radiations

usually have revealed quantitative differences in efficiency rather than qualitative differences in effects. But, there are recorded specific differences in cytological effects, which together with accumulated information on the general characteristics in action between, *e. g.*, x-rays and neutrons on living organisms give reasons for further investigations (*cf.*, *e. g.*, EHRENBERG and NYBOM, 1954).

From a theoretical point of view, but also with regard to the production of mutations for plant breeding purposes it is of great importance to find out whether the distribution of mutation types varies with the kind of irradiation, and especially whether there is a higher proportion of non-destructive or positive mutations induced by any special treatment.

A few such cases on differential mutation induction have been published (*cf.* KÖLMARK, *l. c.*, and MACKEY, *l. c.*). Within our own research group (*cf.* also GUSTAFSSON and NYBOM, 1949) we have drawn attention to the higher frequency of so-called *erectoides* mutations in barley after neutrons (EHRENBERG and NYBOM, 1954). New data on this question have been collected confirming the earlier findings. Altogether, for the years 1950—1954, 82 morphological and physiological barley mutations, selected according to common principles and without regard to the kind of radiation used, may be grouped as follows:

Type of radiation	*Erectoides* mutations	Other viable mutations
Sparsely ionizing: (γ-rays, x-rays and fast protons)	10	39
Densely ionizing: (Fast and thermal neutrons, α-rays)	18	15

χ^2 for the difference in distribution is 10,22 and the corresponding p close to 0,001.

As already mentioned, the question whether genetical changes induced by means of different radiations are characterized by different degrees of viability is also of considerable interest. We are, therefore, accumulating data for studying this in detail, but it will take another few years before the results are ready. The aim of this paper is to describe some other experiments bearing upon this problem.

The effect on viability of chromosome changes induced by x-rays and neutrons. — One of the hypotheses for interpreting the *position*

effect assumes that simultaneously with the chromosome breakage there is also damaged or changed a locus adjacent to the breakage surface, thus leading to a phenotypical effect of the changed chromosome.

Considering that the breakage surfaces produced by different radiations do behave somewhat differently (*e. g.*, with regard to restitution and reunion, *cf.* CATCHESIDE, 1948), it was considered worth while to investigate the effect on viability of chromosome structural changes brought about by different radiations.

In an earlier paper (NYBOM, 1954) the author studied the productivity of a number of barley lines homozygous for x-ray induced chromosome translocations. They had been selected in irradiated material only on sterility indications, excluding such types as were apparently phenotypically deviating (*e. g.*, the chlorophyll change described in the same paper).

It was found that all of the 19 translocation lines tested, with one exception, possessed a next to normal or just slightly changed vigour (measured as the grain yielding ability). The average yielding abilities of all lines was 97,5 % of that of the original line. One might have expected that such a refurnishing of the chromosome structure would impair the well-being more seriously. It is true, however, that many such changes might have been quite lethal and thus eliminated at an early stage.

Now, a new similar material has been collected but this time after neutron irradiation. The neutrons were produced in the 80-cm cyclotron at the Nobel Institute for Physics, Stockholm. An average proton energy of around 3 MeV gave an ionization density of *ca.* 800 ion pairs/μ.[1]

The preparation of the material and the planning of the field trial was analogous with that described in the previous paper (NYBOM, 1954). It should be mentioned, however, that the changes worked with have not been verified cytologically. It is not excluded that some of them might consist of some other structural change, fertile when homozygous but leading to semisterility after crossing with the mother line. But, in analogy with the x-ray material, these sterility causes are in all cases most probably reciprocal translocations.

Eleven cases of neutron induced changes had been identified through crossing with the mother line. For comparison 11 x-ray induced translocations, chosen at random, were inserted into the field trial, alternat-

[1] The same strain of two-row barley (*Hordeum distichum*, var. *Bonus*) was used as before.

TABLE 1. *The relative grain yields of homozygous translocations in barley. Mother variety=100.*

Line	Replication				Average
	I	II	III	IV	
Neutron induced:					
N 2	100	107	56	104	91,8
N 6	135	81	80	109	101,3
N 8	95	85	93	94	91,8
N 12	102	107	104	102	103,8
N 14	97	68	79	83	84,3
N 18	76	117	71	104	92,0
N 20	114	80	111	111	104,0
N 24	125	90	99	110	106,0
N 26	119	75	80	143	104,3
N 30	100	90	85	142	104,3
N 32	89	—	95	—	92,0
				Average of all lines	98,0 ± 2,92
X-ray induced:					
R 9	100	110	112	103	106,3
R 63	79	123	107	120	107,3
R 132	73	93	78	100	86,0
R 141	91	83	119	127	105,0
R 171	112	90	113	158	118,3
R 219	106	73	130	118	106,8
R 246	113	117	125	117	118,0
R 297	63	61	60	63	61,8
R 345	77	92	96	166	107,8
R 360	100	97	91	94	95,5
R 372	120	94	118	89	105,3
				Average of all lines	101,6 ± 3,51

ing with the neutron lines. The mother line was put as each third number, as a standard. All lines were precultivated together the year before in order to obtain uniform seed quality. As in the previous paper, the grain yield of each little plot (*ca.* 205 g) was expressed in % of the neighbouring mother plot. The results are presented in Table 1.

The 11 neutron lines gave an average yield of 98,0 % of that of the mother. None of them, with the possible exception of N 14 with 84,3 %, deviated appreciably from the average.

The yielding ability of the x-ray induced translocations even sur-

passed the mother slightly. The difference was not significant, however, nor was there any certain difference between the two materials.

Of the x-ray translocations, the line R 297 deviated through its low yielding ability, just as it did in the earlier experiments. It is also phenotypically slightly different from the mother line (NYBOM, 1954). It might, thus, constitute the only apparent example of what was mentioned in the beginning of this paragraph, *viz.* a viability-depressing effect somehow following the production of a structural change.

The total impression of the experiment is that it is an exception rather than the rule that structural changes are associated with such phenotypical effects in barley. Moreover, it seems that the breakage surfaces produced by the more densely ionizing neutrons do not deviate from those of the x-rays in this respect.

The viability of x-ray and neutron induced morphological and physiological mutations. — As mentioned in the introduction, neutrons seem to induce a higher proportion of so-called *erectoides* mutations in barley than do x-rays. These mutations are characterized by reduced spike internode lengths, but have usually also increased straw-stiffness and sometimes even better yielding ability. They constitute, thus, an important mutation category from plant breeding point of view.

Now, one may wonder whether the *erectoides* mutations induced by x-rays are somehow different from those originating with higher probability after neutron irradiation. To test this another yield trial was arranged comprising all *erectoides* mutations isolated during the last years (when both x-ray and neutron treatments were performed parallelly). Altogether, there were 7 *erectoides* cases isolated after sparsely ionizing radiations (among which one after γ-irradiation) and 11 after densely ionizing radiations (one after α-irradiation from radon). This trial was arranged in a similar way as the translocation materials. The mother line was used as a standard on each third plot. The yields, expressed in % of that of the mother line, are presented in Table 2.

The yields of different mutants varied from less than 10 % to more than 100 %. However, there seems to be no tendency that the neutron induced mutations are characterized by either especially low or especially high vigour (measured as yielding ability).

Still, another similar material was included in these trials and recorded in Table 2. It consisted of a number of morphological mutations, chosen according to common principles and without regard to origin. Actually, there are certain heterogeneities between the materials coming from x-ray and neutron irradiation; *e. g.*, all cases of *long-awned glumes* were to be found after x-rays whereas all *intermedium-like* mutations had

TABLE 2. *Relative grain yields of morphological and physiological mutations in barley. Mother variety = 100.*

X-ray induced	Yield	Neutron induced	Yield
Erectoides 44	85 %	*Erectoides* 45	117 %
„ 48	49 „	„ 47	90 „
„ 49	87 „	„ 52	50 „
„ 50	50 „	„ 53	75 „
„ 51	11 „	„ 54	48 „
„ 58	46 „	„ 57	6 „
„ 63	11 „	„ 59	35 „
Average of *erectoides*	48,4 ± 12,2 %	„ 60	100 „
		„ 61	10 „
Waxless 9	108 %	„ 62	56 „
„ 10	80 „	„ 64	33 „
„ 11	66 „	Average of *erectoides*	56,4 ± 10,7 %
„ 12	55 „		
Long-awned glumes 4	14 „	Waxless 16	95 %
„ 5	75 „	Intermedium-like 2	102 „
„ 6	59 „	„ 3	31 „
5008	47 „	„ 4	69 „
52018	135 „	Six-rowed 3	83 „
5105	36 „	Irregular spikes 2	33 „
50028	47 „	52092	111 „
52021	88 „	52088	61 „
5001	76 „	Average yield	63,4 ± 7,7 %
Early 4	64 „		
„ 5	82 „		
„ 7	74 „		
„ 8	106 „		
Average yield	64,6 ± 6,3 %		

originated after neutrons. Taken together, the results have some interest, though.

There are no clear differences in viability between those mutations induced by means of x-rays and those coming after neutron irradiation. The total distribution of all analysed morphological and physiological mutants according to grain yield and origin is as follows:

	Grain yield in % of the mother line (*Bonus*).							
	0	20	40	60	80	100	120	140
X-rays, γ-rays	3	1	7	5	5	2	1	
Neutrons, α-rays	2	4	3	3	3	4		

Summing up, the experiments described show that mutants or individuals carrying chromosomal changes induced by densely ionizing radiations, e. g., neutrons, do not differ appreciably with regard to viability from corresponding changes induced by the more common, sparsely ionizing radiations, e. g., x-rays. From practical plant breeding point of view there seems to be no cause to use or to avoid any special kind of irradiation, if not for other reasons, *e. g.*, facility or biological efficiency.

Literature cited

CATCHESIDE, D. G. 1948. Genetic effects of radiations. — Adv. in Genetics 2: 271—358.

EHRENBERG, L. and NYBOM, N. 1954. Ion density and biological effectiveness of radiations. — Acta Agr. Scand. 4: 396—418.

GILES, N. H., DE SERRES, F. J., and PARTRIDGE, C. W. H. 1955. Comparative studies of x ray-induced forward and reverse mutations. — Ann. New York Acad. Sci. 59: 536—552.

GRAY, L. H. 1954. Some characteristics of biological damage induced by ionizing radiations. — Radiation Res. 7: 189—213.

GUSTAFSSON, Å. and NYBOM, N. 1949. Colchicine, X-rays and the mutation process. — Hereditas 35: 280—284.

GUSTAFSSON, Å. and TEDIN, O. 1954. Plant-breeding and mutations. — Acta Agr. Scand. 4: 633—639.

KÖLMARK, G. 1953. Differential response to mutagens as studied by the *Neurospora* reverse mutation test. — Hereditas 39: 270—276.

LEFEVRE, G. JR., RATTY, F. J. JR., and HANKS, G. D. 1953. Frequency of Notch mutations induced in normal, duplicated and inverted x-chromosomes of *Drosophila melanogaster*. — Genetics 38: 345—359.

MACKEY, J. 1954. The biological action of mustards on dormant seeds of barley and wheat. — Acta Agr. Scand. 4: 419—429.

NYBOM, N. 1954. Karyotype and viability in barley. — Acta Agr. Scand. 4: 507—514.

COMPARATIVE IDIOGRAM ANALYSIS OF THE RAT AND THE YOSHIDA RAT SARCOMA

By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

INVESTIGATIONS during the last few years into the chromosome conditions of various ascites tumours of the mouse and the rat have established the fact that the tumour idiograms generally differ from the normal idiogram of the host species. Starting out from the normal chromosome set of the host the tumour gradually adapts its genotype to the existing environment, and this adaptation involves numerical and structural changes of the chromosome set. Since thus the development of tumours from the early pre-cancerous stages on through the progressive stages is reflected in chromosomal changes, it seems very important to define, as closely as possible, in as many cases as possible, the morphological differences in the chromosomes between tumour and host. This may help to visualize the genetic processes involved in the development of the tumours and may eventually bring out rules for mechanisms of the changes. Some regularities are already suggested, as the tendency of mouse tumours to gather in the hypotetraploid chromosome number region, which in this species seems to hold the best promises to high viability for the tumours.

The Yoshida sarcoma of the rat was one of the first to be analysed chromosomally. It belongs to the hypodiploid tumours, which comprise a group of rat and human tumours but which are so far not found in the mouse. The very fact that loss of chromosomes from the diploid set can result in highly viable neoplasms makes this group especially worthy of attention. In the Yoshida tumour the important observation was early made that its stemline held a couple of large metacentric chromosomes, not present among the germline chromosomes of the rat (MAKINO, 1952). The opinions about the relations between the chromosomes of the Yoshida stemline and the normal rat germline have varied considerably and are still quite unsettled. In 1952, MAKINO interpreted the Yoshida stemline as containing »two distinct sets, namely

the set of rod-shaped chromosomes and that of V-shaped ones». The rod chromosomes of the tumour »about 12 in pair» should correspond to 12 pairs of normal rat chromosomes, while the V elements, 8 pairs, should be »of the tumor cell proper» (*l. c.*, p. 22).

Three other Japanese rat tumours, supposedly of independent origin, show striking chromosome similarities to the Yoshida sarcoma. These are the two MTK sarcomas (TANAKA and KANO, 1951) and the Hirosaki sarcoma (MAKINO and KANÔ, 1953). They are hypodiploid like the Yoshida sarcoma, and their stemlines contain large V chromosomes. In the Hirosaki sarcoma a variation was observed in the number of V chromosomes, from one to five; at the same time the rod chromosomes varied from 26—27 to 18—21, thus an increased number of Vs meant a decreased number of rods. This was taken as evidence »that the large V-shaped chromosomes originated by fusion of two large rod-shaped chromosomes at their kinetochore regions» (MAKINO and KANÔ, 1953, p. 1233). A similar mechanism for the origin of the large V chromosomes is assumed by YOSIDA (1955).

After MAKINO had found, in cooperation with HSU (1954), that the normal rat chromosomes are not all telocentric, his earlier opinion of the relations between the two types of idiograms has to be revised. His detailed ideas on this point have not yet been published.

In the present study we have analysed one line of the Yoshida sarcoma, carried in serial transfer by Professor V. EULER at the Institute of Biochemistry, Stockholm. Normal chromosomes of the rat have been studied mainly in spermatogonial cells, but also to some degree in various embryonic tissues and in regenerating liver. Our animals have been obtained from the Institute of Physiology, Lund. They belong to a strain of laboratory rats carried since long at various institutes of Lund University.

The technique employed is the same orcein-squash method as described earlier by us (TJIO and LEVAN, 1954). The work with regenerating liver will be described in detail elsewhere. About 60 % of the liver are extirpated. 24 to 48 hours later 5 to 10 γ colchicine is injected per animal. After 8—12 hours, when the colchicine treatment has effected an accumulation of metaphases, the regenerating tissue is sampled.

We wish to express our sincere thanks to the Swedish Cancer Society for financial support, and to Professor HANS VON EULER and Professor GEORG KAHLSON for the material.

I. THE NORMAL MITOTIC CHROMOSOMES OF THE RAT

Japanese papers up to 1954 describe all rat chromosomes as telocentric, just as the chromosomes are known to be in the mouse (OGUMA, 1935; MAKINO, 1942, 1943). In their study of the sex chromosomes of the rat, KOLLER and DARLINGTON (1934) present a drawing of a testis mitosis with all the chromosomes biarmed: most of the long chromosomes have a small head, most of the small chromosomes are more or less medially attached. GUÉNIN (1948) indicates that all the long chromosomes, except the X, are telocentric; among the rest 17—19 are metacentric.

As a result of the technical progress of later years these contradictory data begin to straighten out. Thus, SACHS (1952, 1953) gives excellent photomicrographs of spermatogonial mitoses, using his modification of the Feulgen-squash technique, and MAKINO and HSU (1954) have made an idiogram analysis of mitoses in embryonic cells, treated according to the hypotonic method of HSU-POMERAT.

If the 20 pairs of autosomes are numbered in falling size order and are divided into three groups according to the location of the centromere, median-submedian (M), subterminal (S) and terminal ones (T), the idiogram of MAKINO-HSU may be represented as follows:

Type	Pair nr	Total pairs
M	11, 12, 15, 16, 19	5
S	1, 4, 8	3
T	2, 3, 5, 6, 7, 9, 10, 13, 14, 17, 18, 20	12

The X-Y pair had the following appearance: X is an S chromosome, in size between 2 and 3, Y is a T chromosome, in size like 20.

A second Japanese analysis of the rat chromosomes has recently been published by YOSIDA (1955). With the same mode of representation his idiogram takes the following shape:

Type	Pair nr	Total pairs
M	12, 13, 16, 18, 19	5
S	1, 2, 5, 10, 14, 17, 20	7
T	3, 4, 6, 7, 8, 9, 11, 15	8

X and Y are both S type, judging from the figure they are of about similar size as in MAKINO-HSU's idiogram.

Even if these two idiograms agree fairly well, considerable differences occur in the detailed interpretation. Both agree that the M chromosomes are found among the smaller ones, both have decided that the

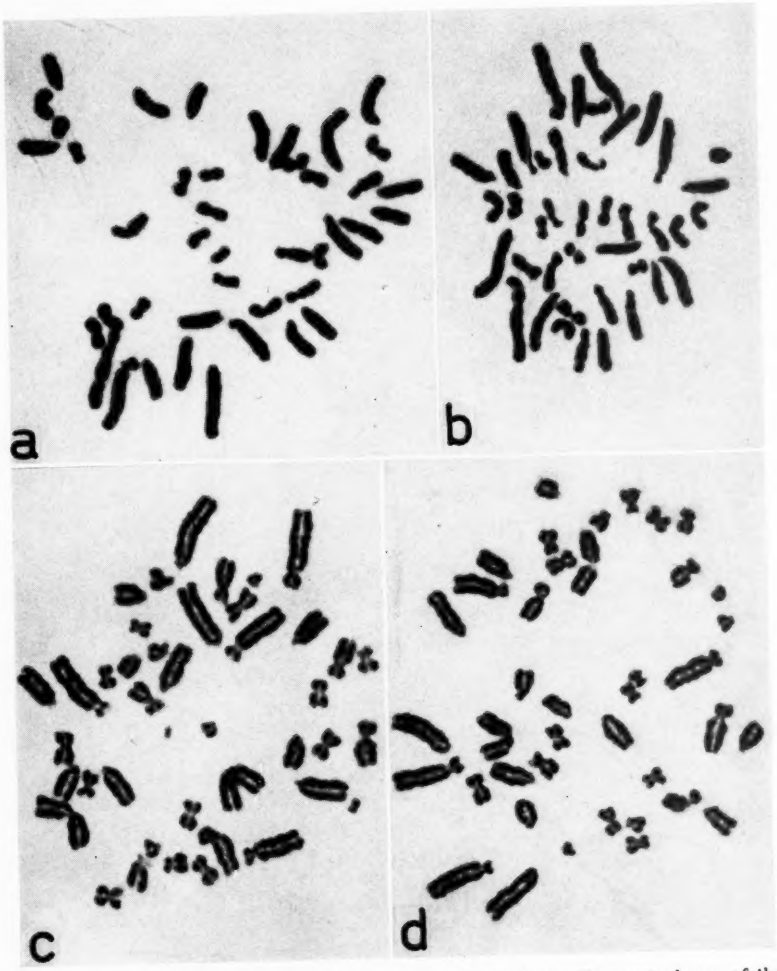


Plate I, *a*, *b*: spermatogonial metaphases of the rat; *c*, *d*: stemline metaphases of the Yoshida sarcoma. — ×2000.

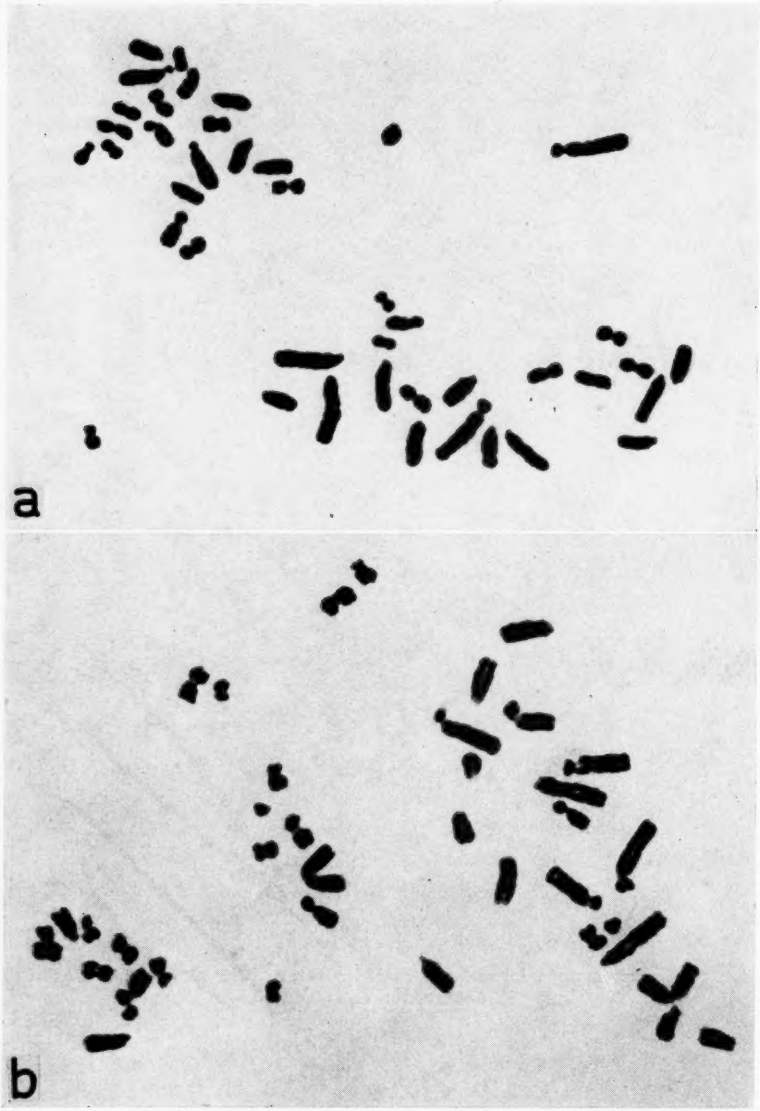


Plate II, *a*: spermatogonial metaphase of the rat; *b*: stemline metaphase of the Yoshida sarcoma. — ×2000.

largest chromosome present is an S type. The idiogram of YOSIDA has a greater number of chromosomes in the S group. Both idiograms have T chromosomes among the smallest ones.

Our result, summarized in the same manner, goes as follows:

Type	Pair nr	Total pairs
M	11, 13, 15, 16, 17, 19, 20	7
S	1, 4, 12, 14, 18	5
T	2, 3, 5, 6, 7, 8, 9, 10	8

X and Y are both T chromosome, X is in size probably between 4 and 5, Y is between 18 and 19. We found all the ten longest autosomal pairs to be the T type, except 1 and 4, which are S. From eleven down, all autosomes are metacentric, 12, 14 and 18 are S, the rest is M.

According to our experience, the classification of the rat chromosomes met with mainly the following difficulties: nr 4 (S) was sometimes provided with such a small head as to simulate a T chromosome; the small S chromosomes (12, 14, 18) may be mistaken for M chromosomes. These difficulties are minimized, if a sufficient number of well-fixed metaphase plates are available.

The rat chromosomes were mainly studied in spermatogonial mitoses (Plate I *a*, *b*, Plate II *a*, Fig. 2 *a*—*c*). In addition, chromosomes were studied from various somatic tissues, the chromosomes from two regenerating liver mitoses being represented in Fig. 1 ; *a* is from a female, and *b* from a male animal. As mentioned above, colchicine treatment preceded the fixation of liver cells, so some of the characteristics of Fig. 1 may be caused by the increased contraction state effected by such a treatment.

As seen from Figs. 1 and 2 *a*—*c*, in which the chromosomes are drawn separately and arranged in the three morphologic groups M, S, and T with the chromosomes of each group in falling size order, the idiogram of the spermatogonial cells and of liver cells agree, generally speaking. We found no aneuploid chromosome number variation in the liver. Except for some tetraploid plates, all analysable plates had exactly 42 chromosomes, deviating numbers could always be attributed to damages at the squashing.

All M chromosomes are small, the longest one is about half the size of the longest T chromosome (2) and about double the size of the smallest M (20). It is impossible to distinguish with certainty between the individual M pairs. The S chromosomes include two long pairs (1, 4) and three small ones (12, 14, 18). The former ones are charact-

Fig. 1. Two idiograms of metaphases in regenerating liver of the rat; *a*: female, *b*: male. — ×2500.

eristic and may always be recognized. The shorter arm of the longest pair (1) is about $^1/_6$ of the whole chromosome. In spermatogonial cells the longer arm of this chromosome exhibits several secondary constrictions, the most proximal one being especially pronounced and generally visible even when the others are concealed. This constriction, as well as other secondary constrictions, were not observed in the liver chromosomes. The second longest S pair (4) has a relatively smaller short arm, measuring about $^1/_8$ of the whole chromosome. The three smaller S pairs may be identified only in especially clear plates. The T chromosomes are, except for the Y, all large chromosomes. The largest one (2) is considerably larger than the others and may always be identified. Nr 3 and 5—10 decrease gradually in length and are not to be identified individually. In spermatogonial mitoses some of them have a secondary constriction, in some cases, as in Fig. 2 *c*, several constrictions may appear on the same T chromosome.

The Y chromosome can be identified with certainty. It is the smallest T chromosome. In spermatogonial mitosis the Y is especially easy to recognize because of its heteropycnosis. It is positively heteropycnotic through resting stage and prophase. At metaphase its contraction is more pronounced than that of the other chromosomes. The Y is seen in Plate I *a* at eleven o'clock, *b* at two o'clock, and in Plate II *a* it is the isolated chromosome at twelve o'clock. In Fig. 2 *a*—*c* it has been drawn separately to the right of the other T chromosomes. Its form is typical: the chromatids are in contact at both ends and bent apart in the middle. Thin chromatic strands run transversely between the chromatids as is

characteristic of c-pairs at late metaphase—telophase. We consider the Y to be at a somewhat more advanced stage than the other chromosomes in spermatogonial cells.

In male liver cells the Y chromosome is readily recognized as the smallest rod chromosome present, but its appearance is less striking than in spermatogonial mitoses. Its heteropycnosis is less obvious, or perhaps missing (Fig. 1 *b*). This differential behaviour of one chromosome in different tissues is of interest and is now being followed up by us.

We have not been able, in mitotic divisions, to determine which one of the T chromosomes is the X. None of them shows heteropycnosis during mitosis. As is evident from meiosis, especially from interkinesis where both X and Y are positively heteropycnotic, X must be one of the longer T chromosomes, probably the fifth longest one, thus the one between pair 3 and 5 (TJIO and LEVAN, 1956). It is not an S chromosome, as suggested by the Japanese authors, quoted above. The four long S chromosomes form autosomal pairs very neatly.

Our study of somatic tissues in the male and the female has confirmed the identity of the smallest sex chromosome as the Y, earlier demonstrated by MAKINO and HSU (1954).

II. THE CHROMOSOMES OF THE YOSHIDA SARCOMA

The chromosome numbers characteristic of various lines of the Yoshida sarcoma have been reported by several authors. Usually a very pronounced chromosome number variation has been observed. Thus, TANAKA and KANÔ (1951) recorded all numbers between 24 and 46, and several scattered numbers above 46. HIRONO and YOKOYAMA (1955) report a variation from 22, with nearly all numbers, to 54, and several higher numbers. In the former case 40 was the most common number (79 of 620 cells), in the latter case 37—39 were most frequent (82 of 396 cells). No individual number was of absolute predominance in the population.

Conditions were quite different in our material (Table 1). We made 422 exact counts in the *s* (stemline) region, 407 of these (96 %) had the number 40, which consequently was quite predominating. Only single cells had other numbers, 39 and 43 constituting the limits of variation for the *s* region. In addition, about 5 % of the cells were 2*s* (double stemline number). In 13 cases the number 80 could be exactly counted in such cells. Some incidence of endoreduplication was observed all

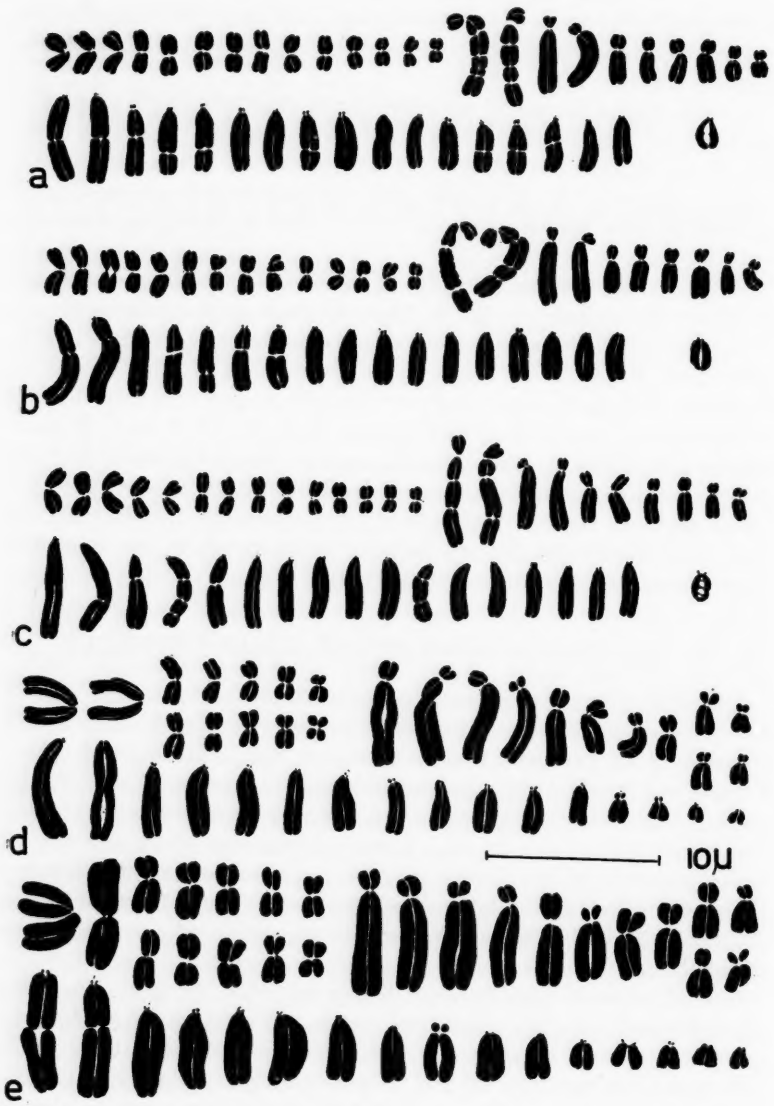


Fig. 2, *a*—*c*: three idiograms of spermatogonial metaphases of the rat; *d*—*e*: two idiograms of stemline metaphases of the Yoshida sarcoma. — ×2600.

TABLE 1. *Chromosome numbers in the Yoshida sarcoma.*

Sample nr	Chromosome number					Total of cells	Estimated numbers	
	39	40	41	42	43		*s*	2*s*
1	2	67	—	4	—	73	115	7
2	—	117	1	1	—	119	110	6
3	2	23	—	—	—	25	112	7
4	—	33	—	1	—	34	100	3
5	1	167	1	1	1	171	100	6
Total	5	407	2	7	1	422	537	29
%	1,2	96,4	0,5	1,6	0,2	100	94,8	5,4

through the slides and is responsible for a low regular frequency of 2*s* cells. Cases of endoreduplication in this tumour are pictured in LEVAN, 1956, Fig. 1.

Photomicrographs of typical stemline metaphases of the Yoshida sarcoma are presented in Plate I *c*, *d* and in Plate II *b*. The aim of the present study was to make as exact a comparison as possible between the rat idiogram and that of the Yoshida tumour. In Fig. 2 *d*, *e* the chromosomes of two cells of the tumour have been drawn and arranged in the same manner as was done in *a*—*c* for the rat chromosomes. In Plate II one representative metaphase plate of each material has been photographed. The chromosomes of both plates are in a similar state of contraction, and give a good comparison between the two materials.

It is seen that a very significant general likeness is present between the normal rat chromosomes and the Yoshida chromosomes. The latter belong to the same three morphologic types, M, S and T. However, very clear differences exist. Thus, the Yoshida chromosomes baffle any attempts to being brought into homologous pairs. This is especially evident with the S chromosomes. Structural rearrangements have blurred the original identity of the rat chromosome pairs. This indicates that a great many changes have taken place during the development of the Yoshida tumour, and not just only the few gross changes which are especially obvious. Our observations immediately disprove YOSIDA's claim: »The chromosomes other than the two large V-shaped elements exactly correspond in morphological characteristics with the corresponding chromosomes in the normal somatic cells of the rat» (YOSIDA, 1955, p. 238).

Even the number of chromosomes in the three morphological groups indicates differences:

Type	Rat	Yoshida
M	14	12
S	10	12
T	18	16

The M group includes ten small chromosomes, which are morphologically similar to the M pairs of the rat, and two large M chromosomes, which have no correspondance among the normal rat chromosomes. These two large M chromosomes are somewhat different in length; they are placed as nr 2 and 4, respectively, in the line of chromosomes, arranged according to falling length (Fig. 3).

The tumour S chromosomes show a variation similar to that of the rat: the longest is nr 1, the shortest is nr 35. Thus, an S chromosome is the longest one in both materials. They are not identical morphologically, however. Nor are there any S chromosomes in the tumour, corresponding morphologically to the second largest S pair of the rat (4).

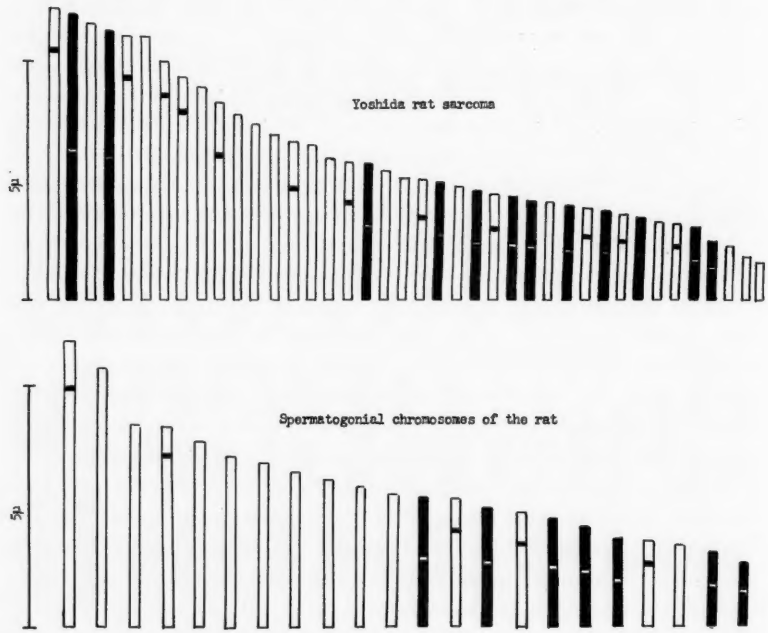


Fig. 3. Diagrams of the metaphase chromosomes of the Yoshida sarcoma stemline (top row), and of the spermatogonial cells of the rat (bottom row). Each diagram is based on ten idiogram analyses.

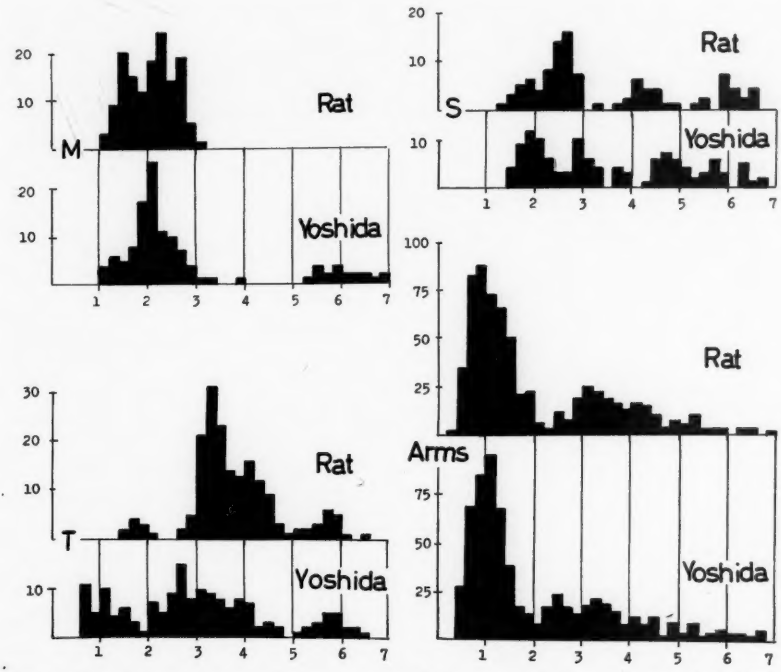


Fig. 4. The distribution of chromosome length in each of the three morphological chromosome classes, and of arm length in the total material. In each case the top histogram represents spermatogonial metaphase chromosomes of the rat, the bottom histogram stemline metaphase chromosomes of the Yoshida sarcoma.

The T chromosomes of the Yoshida show a variation all through the entire length scale. Two of them are definitely longer than the rest, which is a similarity to the T chromosomes of the rat. Then follows a series of eleven T chromosomes in which the length gradually decreases, and finally three minute T chromosomes, smaller than the smallest rat chromosomes. According to YOSIDA (1955), the XY pair may be identified in the Yoshida sarcoma. This is a very important point, and we made every effort to repeat the identification of at least the Y. Since, however, no T chromosome of the right size showed any signs of heteropycnosis, we could not be sure whether any of them corresponds to the Y.

The chromosomes of the rat and of the Yoshida sarcoma were also subjected to a quantitative comparison, based on ten perfectly clear

metaphase plates of each material. In each case every chromosome was drawn under high power in the centre of the viewfield and measured, each chromosome arm by itself. Average lengths for the chromosomes of each ten plates were calculated. The chromosomes of the spermatogonial cells were combined into pairs, while the Yoshida chromosomes were treated individually. The result of these measurements is summarized in Fig. 3, which permits an average comparison between the two materials. Incidental variations within individual plates have been reduced by the procedure employed.

Another comparison, based on the same measurements, is represented by the histograms of Fig. 4. The distribution of all measured chromosome lengths in the three morphological groups was plotted for the rat and for the Yoshida. The values of individual chromosomes in each plate were adjusted to the average chromosome length of the entire material; this was done in order to equal out incidental variations in contraction state between plates. Fig. 4 indicates great similarities between the two materials. In the S group, for instance, the rat histogram forms separate modes for the chromosome pairs 18, 14+12, 4, and 1 (counted from the left). The picture is rather similar for the Yoshida S chromosomes. Within the T chromosomes both materials have a separate mode for the two longest chromosomes (to the outmost right). The most striking dissimilarities are, of course, caused by the long M chromosomes of the Yoshida, forming a separate complex without correspondance in the rat. Furthermore, the Y chromosome forms a small separate mode to the left of the main distribution of the rat T chromosomes, while the Yoshida has a larger separate complex still further to the left, formed by the minute T chromosomes. The rat forms a very high maximum between 3 and 4 μ for the T chromosomes, not present in Yoshida. This missing maximum would, however, be well compensated for, if the arms of the two large M chromosomes were plotted here. They would gather just between 3 and 4 μ. This analysis may indicate in a general way some principles for the changes that have taken place during the development of the Yoshida sarcoma idiogram.

Finally, Fig. 4 includes a comparison of all individual chromosome arms measured. In this histogram the absolute values for measurements have been plotted, since values corrected for the same contraction state were not available. However, plates of fairly similar contraction state had been chosen for this analysis, so the correction would have meant a very insignificant adjustment. The same material is combined in

TABLE 2. *Length distribution of chromosome arms in the rat and in the Yoshida sarcoma.*

Material	Chromosome type	Arm length in μ											Total arms	Mean length
		0,5	1,0	1,5	2,0	2,5	3,0	3,5	4,0	4,5	5,0	5,5 6,0		
Rat	M	10	17	1	—	—	—	—	—	—	—	—	28	1,1
	S	9	3	2	2	—	—	2	—	1	1	—	20	1,7
	T	—	—	1	—	2	6	4	3	—	1	1	18	3,6
	Total	19	20	4	2	2	6	6	3	1	2	1	66	1,9
Yoshida	M	6	13	1	—	2	2	—	—	—	—	—	24	1,4
	S	11	4	2	2	—	1	—	2	1	1	—	24	1,8
	T	2	1	1	2	2	2	3	—	1	—	2	16	3,0
	Total	19	18	4	4	4	5	3	2	2	1	2	64	1,9

Table 2, which presents the arm length distribution in the two average idiograms. A striking agreement is found between the two materials. The maximum frequency of arm length in both is rather low, *viz.* at about one μ. The variation limits are the same (about 0,5—7 μ). It appeared that in the Yoshida the chromosome arms of the T and S chromosomes are on an average somewhat shorter and those of the M chromosomes longer than in the rat. Below 2 μ fall, in the rat, only one T (the Y), 14 S and 28 M arms. Corresponding numbers for the Yoshida are: 4, 17 and 20. It should be noted that the Yoshida has two centromeres and two chromosome arms less than the rat.

In spite of the pronounced similarities in arm length between the two materials, we are convinced that it would not be permissible to conclude that the Yoshida idiogram has developed from the rat mainly by recombinations of entire, unchanged arms, rather than by changes inside the arms. The very method of adding together all arm lengths into one histogram is apt to conceal small differences. Nor is it advisable to propose, as YOSIDA does, the individual four T chromosomes of the rat, which have combined into the large M chromosomes of the Yoshida. Likewise, his continued speculations over this problem seem loosely founded, to put it mildly: »It is likely that such an association between non-homologous chromosomes will accord to them a more or less autonomous tendency in the course of division of the cell and in the differentiation of the tissue, and leading to a malignant growth of the tumor» (YOSIDA, 1955, p. 242).

The ratio long arm : short arm in the S and M chromosomes was studied in both groups of material. All M chromosomes have a ratio

TABLE 3. *The arm index in the S chromosomes of the rat and the Yoshida sarcoma.*

The rat			The Yoshida sarcoma		
Pair nr	Total chromosome length in μ	Arm index	Chromosome nr	Total length in μ	Arm index
1	6,0	5,4	1	6,3	5,7
			5	5,6	5,2
4	4,2	6,9	7	5,1	5,5
			8	4,8	5,1
—	—	—	10	4,2	2,8
			14	3,3	2,6
12	2,8	2,8	17	2,9	2,3
			21	2,6	2,1
14	2,4	2,7	25	2,3	2,1
			30	2,0	2,2
18	1,9	2,6	32	1,9	2,3
			35	1,7	2,3

close to 1 (1,1—1,3). The S chromosomes of both groups fall into two classes: the four longest ones have a ratio of over 5, while the shorter ones have a ratio between 2 and 3. In other words, the arm index is falling with falling chromosome length (see Table 3).

It is of interest that the arm index shows a regularity, which evidently has been maintained through all structural rearrangements occurring at the evolution of the Yoshida idiogram.

A regularity, characteristic of the rat chromosomes, but not kept up at the transition to the tumour idiogram, is the rule that only small chromosomes may have V shape, large chromosomes are either rods or J shaped. It is easy to imagine factors working to this end in the rat idiogram but without significance for the tumour idiogram. Chromosome mechanic conditions during meiosis, for instance, may prevent the exceeding of a certain maximum length for V chromosomes, and such conditions would have no influence in the tumour. A similar, only still more pronounced situation is found in the mouse, where only telocentric chromosomes occur. It may be that all types of metacentrics, including J and small V chromosomes, mean a chromosome mechanic handicap at meiosis in the mouse. In mouse tumours, on the other hand, metacentrics are often found. Similar rules for the chromosome length have been found in the mouse: the chromosomes of this animal form a

length distribution around a maximum, which may be looked upon as representing the optimal chromosome length for this organism. The variation limits for chromosome length set by the mouse, are often exceeded in mouse tumours. Evidently very small and very long chromosomes would mean a chromosome mechanic difficulty at meiosis; they may be erratic or ungainly during pairing and chiasma formation. In a tumour, however, these factors will be of no importance. The passing of the mitotic test is enough for tumour chromosomes.

III. CONCLUDING REMARKS

The comparison made in the present investigation between the chromosomes of normal rat tissues and those of the Yoshida rat sarcoma has aimed to define similarities and dissimilarities between the two materials.

The idiograms belong to the same general type; in both cases the chromosomes are naturally divided into three morphologic classes: M, S and T. Fig. 3, showing an average representation of the chromosomes of the two materials, indicates a good agreement in the curve, expressing the variation in chromosome length from the longest to the shortest chromosome. This agreement was made still more striking, if separate arms are measured, as plotted in the lower right graph of Fig. 4.

An analysis of the arm lengths in the S class gave the following result: both materials were characterized by having four long chromosomes with an arm index of 5—6, and six short chromosomes with an arm index of 2—3. This might indicate that the four longest and six shortest S chromosomes of the tumour have developed from corresponding S pairs of the rat. This leaves us with two intermediate S chromosomes of the tumour, which must have originated from other rat chromosome types.

If such ideas are tried with the M chromosomes, it is possible to derive the ten small M chromosomes of the tumour from five of the seven M pairs of the rat. Among the T chromosomes it should be observed that both the tumour and the rat have two chromosomes decidedly longer than the others. The rat has in addition seven intermediate T pairs+the X, while the tumour has only 11 intermediate T chromosomes. To these, however, should be added the four arms of the two large M chromosomes, which would make the same number of intermediate T chromosomes in the two materials. The Y would then

correspond to the longest one of the remaining four T chromosomes of the tumour, while the origin of the minute T chromosomes of the tumour must be looked for among the small M chromosomes of the rat.

Thus, there is no difficulty to present conjectures about the significance of morphological similarities between the two idiograms under comparison, only we do not think these conjectures very fruitful at the present stage of knowledge.

Some very striking dissimilarities, and others more subtle, exist between the two materials. The number of chromosomes is 42 in the rat, 40 in the tumour; the number of arms is 66 and 64, respectively; the rat has two chromosomes in excess of the tumour of the M and the T class, and two chromosomes less of the S class.

This condition indicates certain regroupings among the arms, as, for instance, two medium-sized T chromosomes of the rat may have fused to the large M chromosomes of the tumour, two T chromosomes+the arms of one M chromosome may have formed the two supernumerary S chromosomes of the tumour, one M chromosome may have been cut up into the minute T chromosomes of the tumour, *etc.* It is clear, however, that other more intricate processes have been at work besides such regroupings. This is seen from the fact that the chromosomes of the tumour cannot be paired into homologous pairs, which is very easily done with the rat chromosomes. The evolution of the sarcoma idiogram has taken place over gross changes, as regroupings of entire arms, and, in addition, over many small structural rearrangements.

The essential difference, inherent in the presence of large M chromosomes in the tumour, has already been discussed above. The absence of such large V chromosomes in the rat has been associated with chromosome mechanic conditions during meiosis. Generally speaking, the counterselection against chromosome morphologic innovations is far more severe in the host organism than in the tumour. This is caused partly by the screening of new chromosomes by the sexual processes, and partly by the greater demands of genotypic balance, of a free-living organism as compared with the parasitic cancer cell. In view of the comparatively uniform and protected environment of the cancer cell, with continuous access to ready-synthetized sources of energy, its evolution may specialize on improved growth rate and division rate. Genetic changes, favouring these functions will mean positive selective value; such changes will accumulate in the cancer cell population, irrespective of whether they would mean sterility or lethality to the germline of the host species. This is why the evolution of tumour stem-

lines is associated with chromosomal alterations, which in abruptness and clumsiness have no counterpart in the evolution of organisms.

None of the Yoshida chromosomes showed heteropycnosis during mitosis. Since this tumour has originated in a male individual, it should have started out with one X and one Y chromosome. Our investigation has shown that both sex chromosomes may become heteropycnotic under certain conditions. This is the case during the first meiotic division from early prophase to, and inclusive, the interkinesis (TJIO and LEVAN, 1956). In spermatogonial mitoses, on the other hand, only Y was heteropycnotic, the X being unrecognizable from the autosomes.

In regenerating liver X showed no signs of heteropycnosis. If Y showed such signs, which we could not decide with certainty, the heteropycnosis of it was anyhow much less pronounced than in the spermatogonial tissue.

It is very suggestive that certain chromosomes or chromosome segments show differential heteropycnosis depending on the tissue, in which the chromosomes exist. Similar cases are known from insects, where in some cases both X and Y are heteropycnotic during meiosis, but only Y in somatic tissue.

Evidently the absence of heteropycnotic sex chromosomes in the Yoshida sarcoma need not mean that these chromosomes are missing. It may just be that the tumour has taken its origin from a tissue, in which the sex chromosomes are isopycnotic with the autosomes, or this property has been developed by the tumour later on.

SUMMARY

Complete idiogram analyses were made for ten spermatogonial metaphases of the rat and ten stemline metaphases of the Yoshida sarcoma. In Fig. 3 these analyses are summarized as two average idiograms. The evolution of the tumour idiogram cannot have taken place only by new-combinations of unchanged chromosome arms. A great number of structural rearrangements, also involving smaller units, must be assumed. The morphologic identity within the originally homologous chromosome pairs has been totally blurred during the development of the tumour.

The sex chromosomes of the rat have been followed during mitosis and meiosis. Both X and Y are heteropycnotic during the first meiotic division, only the Y shows heteropycnosis in spermatogonial mitoses; neither of them is clearly heteropycnotic in liver mitoses. The Y was

identified as the smallest rod chromosome present. The X is one of the larger medium-sized rods, probably number five in size order counted from the longest rod.

Literature cited

GUÉNIN, H. A. 1948. Les hétérochromosomes dans l'ovogénèse des Mammifères. — Journ. Genet. 49: 23—37.

HIRONO, I. and YOKOYAMA, C. 1955. Chromosome features in the original and resistant sublines of the Yoshida sarcoma. — Cytologia 20: 84—88.

KOLLER, P. C. and DARLINGTON, C. D. 1934. The genetical and mechanical properties of the sex-chromosomes. — Journ. Genet. 29: 159—173.

LEVAN, A. 1956. Chromosomes in cancer tissue. — Transact. New York Acad. Sci. (in the press).

MAKINO, S. 1942. Studies on the murine chromosomes. II. Morphological comparison of the chromosomes between the wild form and the domesticated variety of *Rattus norvegicus* BERKENHOUT. — Journ. Fac. Sci. Hokkaido Imp. Univ. 8 (quoted from MATTHEY, 1949).

— 1943. Studies on the murine chromosomes. III. A comparative study of the chromosomes in five species of *Rattus*. — Journ. Fac. Sci. Hokkaido Imp. Univ. 9: 19—57.

— 1952. A cytological study of the Yoshida sarcoma, an ascites tumor of white rats. — Chromosoma 4: 649—674.

MAKINO, S. and HSU, T. C. 1954. Mammalian chromosomes *in vitro*. V. The somatic complement of the Norway rat, *Rattus norvegicus*. — Cytologia 19: 23—28.

MAKINO, S. and KANÔ, K. 1953. Cytological studies of tumors. IX. Characteristic chromosome individuality in tumor strain-cells in ascites tumors of rats. — Journ. Nat. Cancer Inst. 13: 1213—1235.

MATTHEY, R. 1949. Les chromosomes des Vertébrés. — Lausanne, 356 pp.

OGUMA, K. 1935. On the sex chromosomes of rats and mice. — Proc. Jap. Assoc. Adv. Sci. 10 (quoted from MATTHEY, 1949).

SACHS, L. 1952. Polyploid evolution and mammalian chromosomes. — Heredity 6: 357—364.

— 1953. Simple methods for mammalian chromosomes. — Stain Techn. 28: 169—172.

TANAKA, T. and KANÒ, K. 1951. Cytological studies on cancer. IV. General characters of the MTK-sarcomas. New ascites tumors of the rat produced by the administration of azo dye. — Journ. Fac. Sci. Hokkaido Univ. 10: 289—301.

TJIO, J. H. and LEVAN, A. 1954. Some experiences with acetic orcein in animal chromosomes. — Anal. Est. Exper. Aula Dei 3: 225—228.

— and — 1956. Note on the sex chromosomes of the rat during male meiosis. — Anal. Est. Exper. Aula Dei 4: 173—184.

YOSIDA, T. H. 1955. Origin of V-shaped chromosomes occurring in tumor cells of some ascites tumors in the rat. — Proc. Jap. Acad. 31: 237—242.

ON ACCESSORY CHROMOSOMES IN FESTUCA PRATENSIS

IV. CYTOLOGY AND INHERITANCE OF SMALL AND LARGE ACCESSORY CHROMOSOMES

By *NILS OLOF BOSEMARK*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION

ACCESSORY chromosomes in *Festuca pratensis* HUDS. have previously been classified according to their size in root-tip metaphase plates (BOSEMARK, 1954 a). Starting from the dominating type, the *standard accessory chromosome* (st. acc.), accessory chromosomes of smaller size, not smaller than half the standard type, were named *small accessory chromosomes* (s. acc.), and still smaller, *very small accessory chromosomes* (v. s. acc.). Finally, accessory chromosomes larger than the standard type were called *large accessory chromosomes* (l. acc.).

In the first two papers of this series the cytology and inheritance of the standard type of accessory chromosomes were presented (BOSEMARK, 1954 a and b).

The present investigation is a study of the structure, origin and transmission of some derivatives of the standard type, carried out mainly with the purpose of getting information about the factors underlying the mechanism of directed non-disjunction at the first pollen mitosis, which is one of the characteristics of the standard type of accessory chromosome in *Festuca pratensis* (BOSEMARK, 1954 a). If a special segment in this chromosome were responsible for the non-disjunction process, it was considered possible to determine, at least roughly, the position of this segment by studying the effects of different deficiencies and deletions in a number of accessory chromosomes of smaller size.

II. MATERIALS AND METHODS

The small accessory chromosomes studied were all originally found in plants collected in natural populations in Sweden. The large accessory chromosome appeared in the progeny after a cross involving two

such plants. The data have been derived from studies of the original plants as well as their progenies after artificial crosses with plants without accessory chromosomes.

The cytological methods, and the technique used in the study of the transmission of the accessory chromosomes, have been described in the author's earlier publications (BOSEMARK, 1954 a and b).

III. THE SMALL ACCESSORY CHROMOSOMES

1. Frequency of small accessory chromosomes

The frequency of plants with small and very small accessory chromosomes in different materials is given in Table 1.

TABLE 1. *Frequency of small and very small accessory chromosomes in spontaneous and experimental material of Festuca pratensis.*

Material	Number of plants with respective types of accessory chromosomes			Average number of st. acc.	% plants with s. or v. s. acc.
	Only st. acc.	Only s. or v. s. acc.	St. acc. + s. or v. s. acc.		
Spontaneous plants from Sweden	528	2	2	1,70	0,75
Crosses involving plants with one and two st. acc.	2096	3	1	1,39	0,19
Crosses involving plants with more than two st. acc.	1418	—	15	5,97	1,05

As seen, plants with accessory chromosomes of smaller types are seldom found in wild populations. It is, however, obvious that these chromosomes originate from the standard type, but that the production either is very low, or that most derivatives are immediately eliminated. As pointed out earlier (BOSEMARK, 1954 b), in the progenies after artificial crosses the number of plants with smaller types of accessory chromosomes increases with an increasing number of standard accessory chromosomes in the mother plants. This is to be expected from mere numerical reasons, but may also partly depend on the corresponding increase in cytological irregularities during meiosis and pollen mitosis (BOSEMARK, 1954 a).

2. The small accessory chromosome from plant 926

The chromosome number of the original plant was $2n=14+1$ s. acc. From Fig. 1 it is seen that the size of this accessory chromosome is about half that of the standard type. The size as well as the shape indicates a terminal centromere and loss of one arm. The transmission in somatic divisions in root-tips is regular. In Table 2 a summary is given of the results of three reciprocal crosses involving this small accessory chromosome. Two of the crosses were performed with plant 926 as the one parent, in the third one a progeny plant from the first type of cross took part.

TABLE 2. *Summary of the results from three reciprocal crosses involving the small accessory chromosome from plant 926.*

Parents. Number of acc. chr. ♀ ♂	F_1 Number of acc. chr. 0	1 s.	2 s.	Number of plants	M. s. acc. chr. per plant
1 s. × 0	37	7	2	46	0,24
0 × 1 s.	73	29	—	102	0,28

The number of plants in the individual crosses is small, but the results are quite consistent; in no case do the data indicate that the small accessory chromosome has undergone non-disjunction on the male side. This was further verified by studies of more than 100 anaphases of the first pollen mitosis. In none of these were seen lagging accessory chromosomes between the two anaphase groups. Thus, it is obvious that this small accessory chromosome has lost something essential for the process of non-disjunction. The meiotic elimination, as reflected by the results of the crosses, is much higher than is usually the case in plants having one standard accessory chromosome.

Attempts were now made to analyse this small accessory chromosome at pachytene, and in this way to determine which arm had been lost. As seen from Fig. 20 (see also BOSEMARK, 1954 a), the standard type of accessory chromosome has a characteristic morphology at pachytene. The right arm mainly consists of a large heterochromatic segment, sometimes with a small tail or knob-like formation at the distal end. The left arm has three heterochromatic blocks, which are generally easy to distinguish, the one in the middle being the largest. Finally, the distal end of this arm has a conspicuous arrow-shaped formation, which

makes it easy to distinguish from the right arm. Here, it should be pointed out that the morphology of the standard type now described is consistent with only one stage of contraction, and that at earlier stages the heterochromatic blocks can be seen to consist of smaller units. At later stages, on the other hand, also the segments in the left arm approach so close to each other that they cannot be separated (Figs. 10 and 17). The stage of contraction figured here has been chosen to illustrate the structure of the standard accessory chromosomes simply because at this stage good slides with fairly well differentiated chromosomes are most easily obtained.

As previously mentioned (BOSEMARK, 1954 a), univalent standard accessory chromosomes in *Festuca pratensis*, just as univalent B-chromosomes in maize (MCCLINTOCK, 1933) and the deficiency fragment in rye (MÜNTZING and LIMA-DE-FARIA, 1952), have a tendency to pair with themselves at pachytene. The univalent small accessory chromosomes from plant 926 regularly showed such fold-backs. This rendered the analysis more difficult, and made it necesssary to look for fairly early stages. In all observable cases, however, the characteristic arrow-shaped end of the left arm was present, and in most cases also the three main segments of this arm could be recognized (Figs. 2—4). Thus, there is no doubt that the small accessory chromosome has lost the right arm with the large heterochromatic segment. Where the break has taken place in the primary constriction could not be decided, and therefore it is not known whether the small accessory chromosome has a complete centromere or not. As it is very likely that besides special segments in one or both arms, the centromere also plays a rôle in the functioning of the non-disjunction mechanism, it could not be decided whether the failure of this activity was due to the loss of the right arm, or to a part of the centromere, or to a combination of both these events. The complex structure of the centromere, manifested by the works of LIMA-DE-FARIA (1949, 1952, 1955), among others, makes it likely that breaks in the centromeric region can give rise to products with widely different centromeric potency.

The behaviour of the small accessory chromosome at later stages of meiosis did not markedly deviate from that of the standard type. Thus, when situated between the poles at mid-anaphase I, the small accessory chromosome regularly divides. As judged from studies of telophase I, the elimination at this stage is very slight. On the other hand, a marked elimination could be seen to take place at anaphase II. At the tetrad stage, micronuclei were also much more common than is usually the

case in plants with a single standard accessory chromosome. This is in conformity with the results of the crosses, which showed a marked loss of this small accessory chromosome.

3. The small accessory chromosome from plant 2311

This plant had the chromosome number $2n=14+2$ st.$+1$ s. acc. In root-tip metaphase plates the appearance of the small accessory chro-

TABLE 3. *Results from a cross involving the small accessory chromosome from plant 2311.*

A. Observed chromosome numbers.

Parents. Number of acc. chr.	F_1 Number of acc. chr.								Number of plants
	1 st.	2 st.	3 st.	1 s.	1 st. + 1 s.	2 st. + 1 s.	2 st. + 2 s.	3 st. + 2 s.	
(2 st. + 1 s.) × 0	10	2	1	—	8	1	—	1	23
0 × (2 st. + 1 s.)	1	13	—	2	—	—	16	—	32

B. Analysis of the inheritance of the standard type.

Parents. Number of acc. chr.	F_1 Number of st. acc. chr.				Number of plants	M. st. acc. chr. per plant
	0	1 st.	2 st.	3 st.		
(2 st. + 1 s.) × 0	—	18	3	2	23	1,30
0 × (2 st. + 1 s.)	2	1	29	—	32	1,84

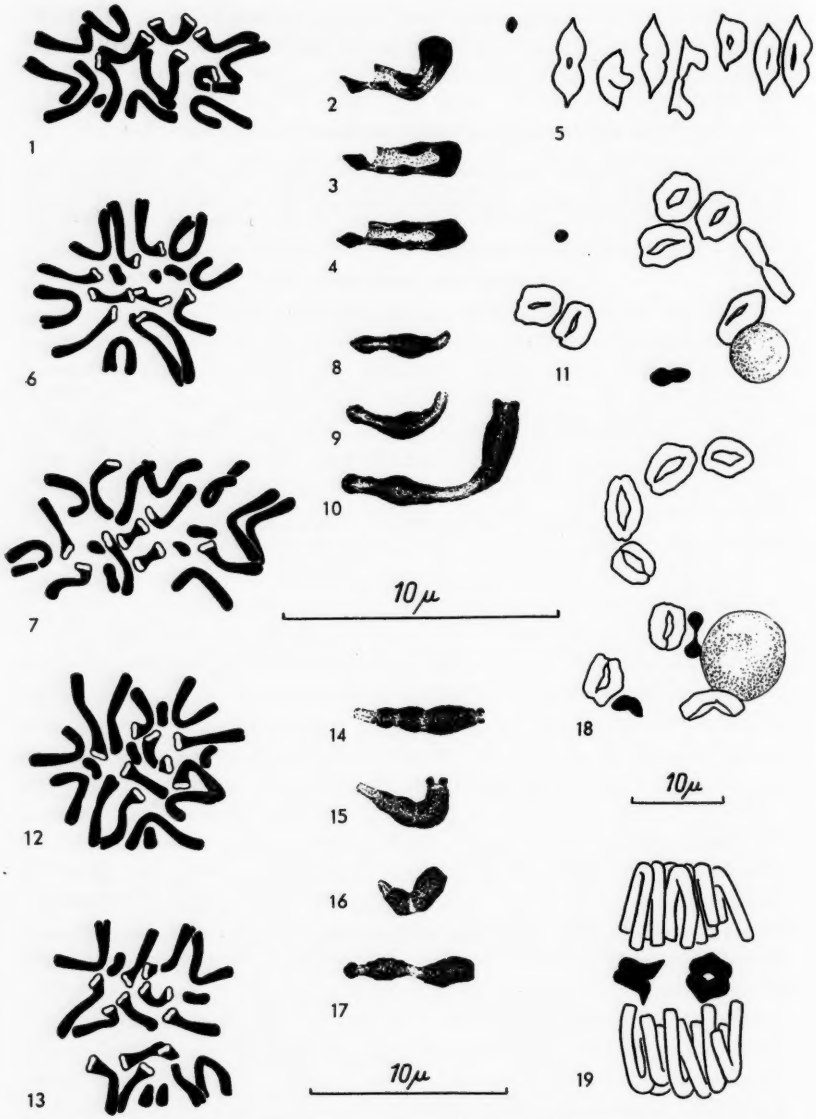
C. Analysis of the inheritance of the small type.

Parents. Number of acc. chr.	Number of s. acc. chr.			Number of plants	M. s. acc. chr. per plant
	0	1 s.	2 s.		
(2 st. + 1 s.) × 0	13	9	1	23	0,48
0 × (2 st. + 1 s.)	14	2	16	32	1,06

D. Functioning of the non-disjunction mechanism of the small accessory chromosome in relation to absence and presence of standard accessory chromosomes in the same pollen grain.

Types of acc. chrs. in the pollen	Cases of disjunction	Cases of non-disjunction
Only s. acc.	2	0
st. + s. acc.	0	16

$p = 0{,}0065$



Figs. 1—5. The small accessory chromosome from plant 926. — Fig. 1. Root-tip metaphase plate with $2n=14+1$ s. acc. — Figs. 2—4. Fold-backs formed by univalent s. acc. chrs. at early pachytene. — Fig. 5. Metaphase I in a plant with $2n=14+1$

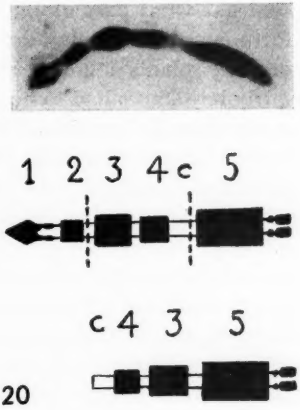


Fig. 20. Photograph of paired standard accessory chromosomes at pachytene, and schematic drawings showing how the small accessory chromosome from plant 373 has originated through an inversion in combination with a deficiency. The dotted lines indicate the positions of the breaks. The large accessory chromosome is an iso-chromosome, which has originated through misdivision in the centromere of the inversion-deficiency-chromosome. c=centromere.

mosome is quite the same as that from plant 926 (Figs. 6 and 7). In 1954 the original plant was crossed to a plant lacking accessory chromosomes, and the transmission of the accessories studied in both directions (Table 3).

In the first part of the table the distribution of the observed chromosome numbers in the progeny is given. In the two following parts the inheritance of the standard type and the small type has been treated separately. As judged from these separate analyses, there are no pronounced differences in behaviour between the two types. The non-disjunction mechanism has functioned regularly in both cases, giving marked differences between the reciprocal crosses with respect to the average number of accessory chromosomes per plant in the progeny.

Thus, this small accessory chromosome still seems to have its capacity for non-disjunction unaffected. It was therefore of special interest to

s. acc. — Figs. 6—11. The small accessory chromosome from plant 2311. — Fig. 6. Root-tip metaphase plate from plant 2311 with $2n=14+2$ st.$+1$ s. acc. — Fig. 7. The same from a progeny plant with $2n=14+2$ st.$+2$ s. acc. — Figs. 8, 9. Univalent s. acc. chrs. in plant 2311 at late pachytene. The univalents have already reduplicated. — Fig. 10. Paired st. acc. chrs. from the same plant and at the same stage of contraction. — Fig. 11. Diakinesis in plant 2311. — Figs. 12—19. The small accessory chromosome from plant 373. — Fig. 12. Root-tip metaphase plate from plant 373 with $2n=14+4$ st.$+1$ s. acc. — Fig. 13. Root-tip metaphase plate from a progeny plant with $2n=14+2$ st.$+2$ s. acc. — Figs. 14, 15. Paired s. acc. chrs. at pachytene. — Fig. 16. Paired s. acc. chrs. at pachytene-diakinesis from a plant with $2n=14+2$ st.$+$ $+2$ s. acc. — Fig. 17. Paired st. acc. chrs. from the same plant. — Fig. 18. Diakinesis in a plant with $2n=14+2$ st.$+2$ s. acc. — Fig. 19. The first pollen mitosis from the same plant.

find out what part of the chromosome was missing in this case. Unfortunately, the pachytene stage in the mother plant as well as in the progeny plants studied was very unsuitable for analysis. Therefore fairly late stages had to be chosen for the study, the chromosomes being much less differentiated than in plant 926. However, the previously mentioned characteristics of the right arm of the standard type made it possible to establish that also in the small accessory chromosome from plant 2311, just as in the one from plant 926, it is the right arm that is missing. This can be seen in Figs. 8—10, where, besides two small univalent accessory chromosomes, a bivalent of the standard type, from the same plant and at the same stage of contraction, has been included for comparison. The small accessory chromosomes have already reduplicated, being very similar to bivalents. Fold-backs of the same type as those seen in plant 926 were also present, but owing to the late stage they were less useful for the present purpose.

The question now arises as to what is the difference between the two small accessory chromosomes, one of which has completely lost its ability to undergo non-disjunction, whereas the other seems to be unaffected. The difference might of course be in the centromeric region, and the low meiotic elimination of the small type in plant 2311, in contrast to that of 926, speaks in favour of such an explanation. There are, however, other possibilities to explain the difference. In the cross direction, where the plant with acccessory chromosomes has been the pollen parent, there are two plants which have one small accessory chromosome only. These plants should consequently have arisen through functioning of pollen grains that received only one small accessory chromosome during meiosis. The plants which have two small accessory chromosomes, showing that the non-disjunction mechanism has functioned, have all, in addition, two standard accessory chromosomes. Similarly, these plants must be the result of functioning of pollen grains, which, besides the small accessory chromosome, have received at least one accessory chromosome of the standard type. Thus, if the functioning of the non-disjunction mechanism of the small accessory chromosome is considered in relation to absence and presence of standard accessory chromosomes in the pollen grains, the data can be arranged as in Table 3 D.

In spite of the low numbers, it is unlikely that the proportions 2 : 0 and 0 : 16, represent the same distribution. A statistical analysis according to the method in BONNIER and TEDIN (1940; pp. 226—228) gives a p-value of $0{,}0065$. Thus, it is very likely that the presence of standard

accessory chromosomes in the pollen grains is essential for a successful non-disjunction of this small accessory chromosome.

The study of the other stages of meiosis did not reveal anything deviating from the normal behaviour of accessory chromosomes in *Festuca pratensis*. The frequency of tetrad micronuclei was fairly low, which is in conformity with the results of the crosses.

4. The small accessory chromosome from plant 373

The chromosome number of this plant was $2n=14+4$ st.$+1$ s. acc. As seen from Figs. 12 and 13, the small accessory chromosome is only slightly shorter than the standard type. The centromere was first considered to have its normal position, only a small portion of one of the arms being missing. As was found later on, the centromere actually has a terminal position.

In a cross involving the original plant, not only progeny plants were obtained with a varying number of accessory chromosomes of the small and standard types, but also two plants with a new, large type of acessory chromosome, which will be described in detail in the next section of this paper. Thus, the present plant was also studied to find an explanation for the origin of this chromosome.

Using progeny plants from the first cross, new crosses of the type $(14+2$ st.$+1$ s. acc.$)\times 14$ and reciprocally were carried out in 1954. The results of three crosses of this type have been summarized in Table 4.

The separate analyses of small and standard accessory chromosomes do not indicate any marked differences in the mode of transmission between the two types. The mechanism of non-disjunction, however, has on the whole functioned better in the small than in the standard type. As was done for plant 2311, the frequency of non-disjunction in pollen grains with only small accessory chromosomes can be compared to that in pollen grains having standard as well as small accessory chromosomes (Table 4 D). The proportions of disjunction to non-disjunction are 3 : 2 and 8 : 26, respectively. The probability of these two proportions representing the same distribution is 0,1252. Thus, there is a slight tendency to a similar positive influence of the standard type on the non-disjunction of the small, as was observed in plant 2311. It is, however, evident that even when present alone in the pollen grain, this small accessory chromosome is capable of undergoing non-disjunction. If we consider the functioning of the non-disjunction mechanism of the standard type in relation to absence and presence of small accessory

TABLE 4. *Summary of the results from three reciprocal crosses involving the small accessory chromosome from plant 373.*

A. Observed chromosome numbers.

Parents. Number of acc. chr. ♀ ♂	F_1 Number of accessory chromosomes										Number of plants
	0	1 st.	2 st.	3 st.	4 st.	1 s.	2 s.	1 st. + 1 s.	2 st. + 1 s.	2 st. + 2 s.	
(2st.+1s.)×0	2	38	3	1	—	3	—	25	—	—	72
0×(2st.+1s.)	44	21	22	1	1	3	2	7	1	26	128

B. Analysis of the inheritance of the standard type.

Parents. Number of acc. chr. ♀ ♂	F_1 Number of st. acc. chr.					Number of plants	M st. acc. chr per plant
	0	1	2	3	4		
(2 st. + 1 s.) × 0	5	63	3	1	—	72	1,00
0 × (2 st. + 1 s.)	49	28	49	1	1	128	1,04

C. Analysis of the inheritance of the small type.

Parents. Number of acc. chr. ♀ ♂	F_1 Number of s. acc. chr.			Number of plants	M. s. acc. chr. per plant
	0	1	2		
(2st.+1s.)×0	44	28	—	72	0,39
0×(2st.+1s.)	89	11	28	128	0,62

D. Functioning of the non-disjunction mechanism of the small accessory chromosome in relation to absence and presence of standard accessory chromosomes in the same pollen grain.

Types of acc. chr. present in the pollen	Cases of disjunction	Cases of non-disjunction
Only s. acc.	3	2
st. + s. acc.	8	26

$p = 0{,}1252$

E. Functioning of the non-disjunction mechanism of the standard accessory chromosome in relation to absence and presence of small accessory chromosomes in the same pollen grain.

Types of acc. chr. present in the pollen	Cases of disjunction	Cases of non-disjunction
Only st. acc.	22	25
s. + st. acc.	7	27

$\chi^2 = 5{,}901$; $0{,}02 > p > 0{,}01$.

chromosomes in the pollen grains, the proportions of disjunction to non-disjunction in the two cases are 22 : 25 and 7 : 27, respectively (Table 4 E). This gives a $\chi^2 = 5{,}901$ with a p-value between 0,02 and 0,01. Thus, it is rather likely that the presence in the pollen grains of these small accessory chromosomes together with the standard type increases the proportion of non-disjunctions of the latter.

The different stages of meiosis were studied in plants having 2 st.+ +2 s. acc. At late pachytene a number of paired small accessory chromosomes could be analysed (Figs. 14—16). These analyses showed that the centromere has a terminal position, and further that a part of the left arm of the standard accessory chromosome has been lost. Fig. 20 illustrates how this has probably happened. In a standard accessory chromosome two breaks have taken place: one in the centromeric gap between the segments 4 and 5, the other probably between the segments 2 and 3. Segments 1 and 2 have then been lost at the same time as segment 3—4 has been inverted. That the centromere actually has a terminal position is obvious also from the study of anaphases of the first pollen mitosis (Fig. 19). In contrast to the standard accessory chromosomes, which usually form bivalents at pachytene, when present in duplicate — and did so also in these plants — the small accessory chromosomes instead often paired with themselves. Why the frequency of such intra-arm pairing is higher in the small than in the standard type is not known. One reason might be that the small accessory chromosome is relatively more heterochromatic than the standard type, and that the two largest heterochromatic blocks are situated close to each other, not being separated by the centromeric gap. The frequent intra-arm pairing at pachytene results in a high frequency of univalents at diakinesis and metaphase I. At metaphase I the difference in size between the two types of accessory chromosomes was so slight that a separation was impossible. At diakinesis, however, the size differences were greater, and the two types of bivalents had, moreover, a slightly different appearance. Thus the standard accessory chromosomes, as is usually the case, appeared as fairly compact dumb-bell-shaped formations, whereas the small accessory chromosomes were more loosely associated, often being only held together by a thin thread (Fig. 18). When two univalents were present, the bivalent was practically always of the first-mentioned type. Only in a few cases higher associations between small and standard accessory chromosomes were seen. This speaks strongly in favour of the idea that most of the univalents seen at metaphase I were of the small type. The frequency of different configurations at diakinesis

TABLE 5. *Pairing behaviour at diakinesis and metaphase I in a plant with 2n=14+2 st.+2 s. acc.*

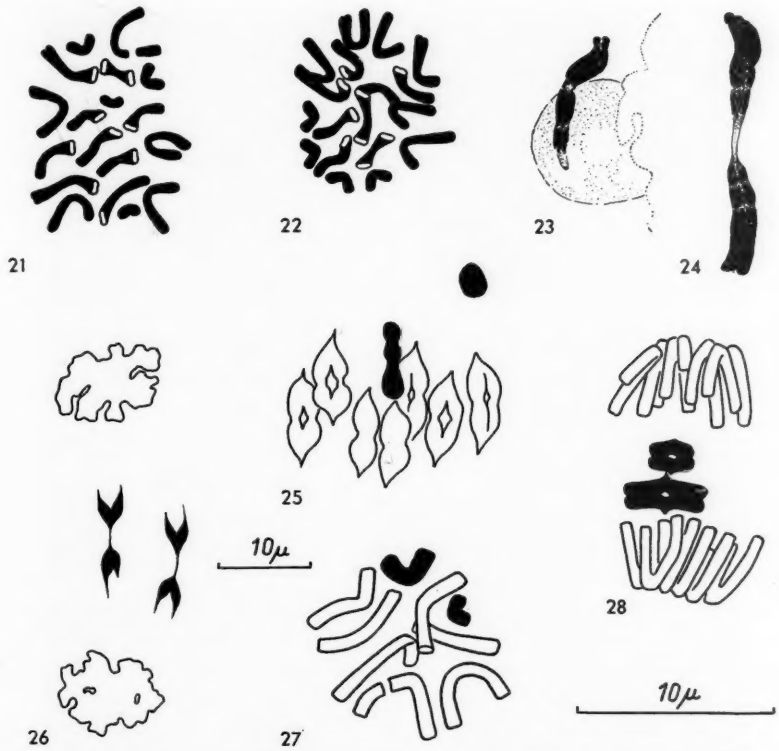
Configurations	Number of cells studied at	
	Diakinesis	Metaphase I
1 $st._{II}$ + 1 $s._{II}$	34	2
1 $st._{II}$ + 2 $s._{I}$	33	35
2 $st._{I}$ + 1 $s._{II}$	4	15
2 $st._{I}$ + 2 $s._{I}$	2	—
1 $hetero_{IV}$	10	—
1 $hetero_{III}$ + 1 $s._{I}$	1	—
1 $hetero_{II}$ + 1 $st._{I}$ + 1 $s._{I}$	1	—
Total	85	52

and metaphase I in a plant with $2n=14+2$ st.+2 s. acc. is given in Table 5.

If the classifications above are correct, it probably means that the small accessory chromosomes, even when present as bivalents at pachytene, seldom form chiasmata. This might then depend on the same factors which reduced the degree of inter-chromosome pairing, namely, the relatively higher content of heterochromatin in this derivative. The frequency of micronuclei in the tetrads was much lower than could be expected from the results of the crosses. The plants studied cytologically, however, were not those crossed, and different plants often differ very much with respect to the frequency of tetrad micronuclei.

IV. THE LARGE ACCESSORY CHROMOSOME

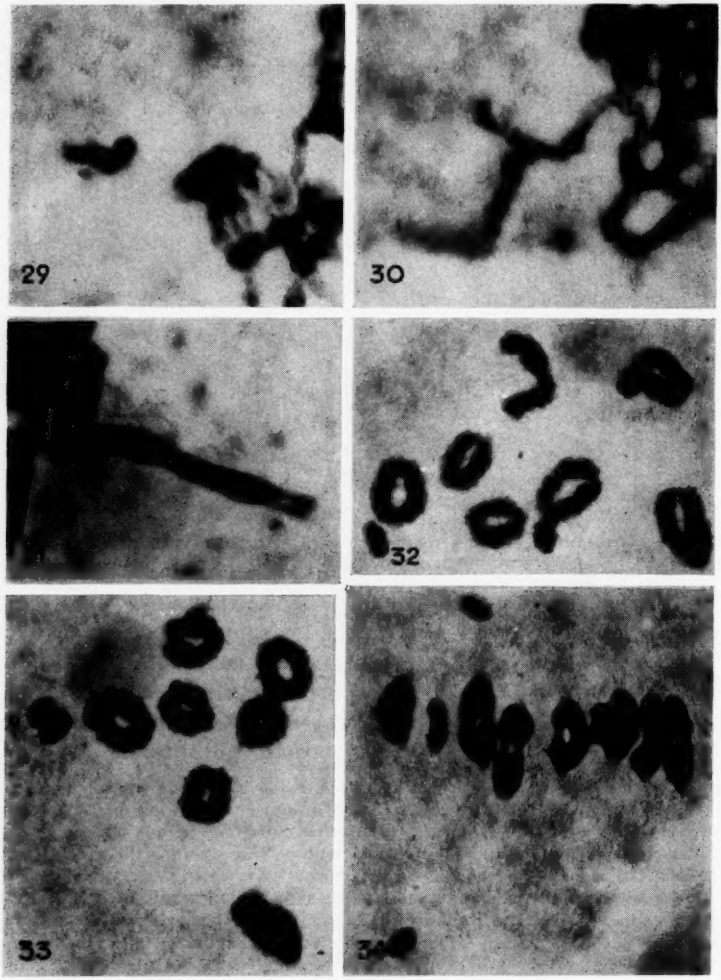
As already mentioned, two plants with accessory chromosomes of about double the size of the standard type were found in the progeny from plant 373. The appearance of the large accessory chromosome in root-tip metaphase plates is seen in Figs. 21 and 22. The original plants had the chromosome numbers $2n=14+2$ st.+1 l. acc. and $2n=14+$ +2 st.+2 l. acc. In the summer of 1953 the plant with two large accessory chromosomes was crossed to a plant lacking accessories. The result of this cross is given in Table 6. These data showed that the large accessory chromosome possesses about the same ability to undergo nondisjunction as the standard type, but that the meiotic elimination is probably a little higher. As the number of progeny plants was small, a



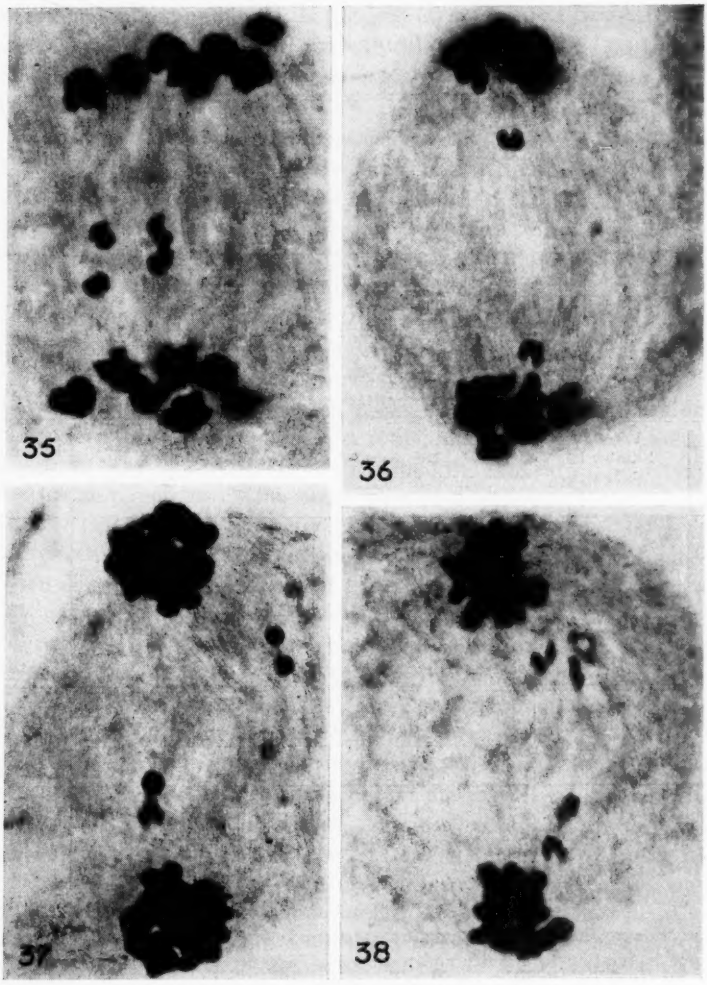
Figs. 21—28. The large accessory chromosome. — Fig. 21. Root-tip metaphase plate from a plant with $2n=14+2$ st.$+2$ l. acc. — Fig. 22. The same from a plant with $2n=14+4$ l. acc. — Fig. 23. A large accessory chromosome showing inter-arm pairing at pachytene. — Fig. 24. Two large accessory chromosomes at late pachytene showing inter-chromosome pairing. — Fig. 25. Metaphase I in a plant with $2n=14+2$ st.$+$ $+2$ l. acc.; one hetero-trivalent and one univalent l. acc. — Fig. 26. Anaphase I in a plant with $2n=14+2$ l. acc. Dividing univalents showing poleward movement of chromosome ends. — Fig. 27. Metaphase plate from the first pollen mitosis in a plant with $2n=14+2$ st.$+2$ l. acc. — Fig. 28. Anaphase of the first pollen mitosis in the same plant.

new cross was made in the summer of 1954, using as the one parent a progeny plant with 2 st.$+2$ l. acc. from the previous cross. Also the results of this cross are given in Table 6.

The loss of accessories of both types is about the same in the two years. The non-disjunction mechanism of the standard type has functioned relatively poorly in both years, and seems to be inferior to



Figs. 29—30. Fold-backs formed by univalent small accessory chromosomes from plant 926 at early pachytene. — Fig. 31. Large accessory chromosome showing inter-arm pairing at pachytene. — Fig. 32. Diakinesis in a plant with $2n=14+2$ l. acc. The l. acc. chrs. are present as univalents, probably due to inter-arm pairing. — Fig. 33. Another cell from the same plant. The l. acc. chrs. are here associated. — Fig. 34. Metaphase I in a plant with $2n=14+2$ st. $+2$ l. acc.; 1 st.$_{II}+2$ l.$_{I}$ — Figs. 29—30, 2800×; Fig. 31, 2670×; the others 1550×.



Figs. 35—38. The appearance of dividing univalent large and standard accessory chromosomes at anaphase I. — Fig. 35. From a plant with $2n=14+2$ l. acc. — Fig. 36. From a plant with $2n=14+2$ st.$+2$ l. acc. The univalent which has divided is of the large type. — Fig. 37. From a plant with $2n=14+2$ st.$+2$ l. acc. The dividing univalent to the left is of the large type, the other is of the standard type. In the latter one it can be seen that also in the standard type the ends are directed towards the poles. — Fig. 38. From a plant with $2n=14+2$ l. acc. One of the dividing univalents is out of focus (1550×).

TABLE 6. *Results of two reciprocal crosses involving the large accessory chromosome.*

A. Observed chromosome numbers.

Year	Parents. Number of acc. chr. ♀ ♂	F_1 Number of accessory chromosomes													Number of plants
		0	1 st.	2 st.	3 st.	1 l.	2 l.	4 l.	1 st. + 1 l.	1 st. + 2 l.	2 st. + 1 l.	2 st. + 2 l.	2 st. + 3 l.	4 st. + 2 l.	2 st. + 4 l.	
1953	(2 st. + 2 l.) × 0	1	4	2	—	1	1	—	14	3	1	—	—	—	—	27
	0 × (2 st. + 2 l.)	6	5	5	1	2	5	—	1	1	—	6	—	1	—	33
1954	(2 st. + 2 l.) × 0	16	8	8	—	1	2	1	18	6	2	6	—	—	1	69
	0 × (2 st. + 2 l.)	24	19	14	—	3	5	1	4	2	2	20	1	—	3	98

B. Analysis of the inheritance of the standard type.

Year	Parents. Number of acc. chr. ♀ ♂	F_1 Number of st. acc. chr.					Number of plants	M. st. acc. chr. per plant
		0	1	2	3	4		
1953	(2 st. + 2 l.) × 0	3	21	3	—	—	27	1,00
	0 × (2 st. + 2 l.)	13	7	11	1	1	33	1,09
1954	(2 st. + 2 l.) × 0	20	32	17	—	—	69	0,96
	0 × (2 st. + 2 l.)	33	25	40	—	—	98	1,07

C. Analysis of the inheritance of the large type.

Year	Parents. Number of acc. chr. ♀ ♂	F_1 Number of l. acc. chr.					Number of plants	M. l. acc. chr. per plant
		0	1	2	3	4		
1953	(2 st. + 2 l.) × 0	7	16	4	—	—	27	0,89
	0 × (2 st. + 2 l.)	17	3	13	—	—	33	0,88
1954	(2 st. + 2 l.) × 0	32	21	14	—	2	69	0,83
	0 × (2 st. + 2 l.)	57	9	27	1	4	98	0,71

D. Functioning of the non-disjunction mechanism of the standard accessory chromosome in relation to absence and presence of large accessory chromosomes in the same pollen grain.

Year	Types of acc. chr. present in the pollen	Cases of disjunction	Cases of non-disjunction
1953	Only st. acc.	6	6
	l. + st. acc.	2	8
1954	Only st. acc.	19	14
	l. + st. acc.	6	26

χ^2 1954 year's data = 10,347; $p \approx 0{,}001$

χ^2 total = 12,298; $p < 0{,}001$

that of the large accessory chromosome. If the data from the two years are put together, the proportions of disjunction to non-disjunction for the standard type and the large type are 33 : 54 and 13 : 49, respectively. This gives a $\chi^2 = 4{,}881$, with a p-value between 0,05 and 0,02. As will be described later on, it is likely that quite a number of the plants with two large accessory chromosomes have originated through irregular distribution of these chromosomes at anaphase I. Thus, even if the data had been statistically more significant, it could not have been said with certainty that there are any differences between the two types with respect to their ability to undergo non-disjuncion.

In Table 6 D an analysis is given of the non-disjunction frequency of the standard accessory chromosome in pollen grains which have had only one such chromosome, compared to that in pollen grains which have carried one standard plus one or two large accessory chromosomes. The proportions of disjunction to non-disjunction have been calculated in the same way as described earlier. When the standard type is present alone in the pollen grain, the proportion of disjunction to non-disjunction is 19 : 14, whereas when large accessory chromosomes are present together with the standard type, it is 6 : 26. The percentages of successful non-disjunctions are 42,4 and 81,3, respectively. This difference is highly significant and gives a $\chi^2 = 10{,}347$ with a p-value very close to 0,001 ($p = 0{,}001$; $\chi^2 = 10{,}827$). The same tendency, though not significant, is present in the cross from 1953, the corresponding figures being 6 : 6 and 2 : 8. Taken together, the data from the two crosses give a $\chi^2 = 12{,}298$ with a p-value smaller than 0,001. Thus, there is no doubt that the presence of large accessory chromosomes together with the standard type, in the same pollen grain, affects the standard type in such a way as to make it more liable to undergo non-disjunction.

The cytology of the large type has been studied in plants with a varying number of large as well as standard accessory chromosomes, and will now be described in more detail. At pachytene, in a plant with two large accessory chromosomes, these were generally present as two separate bodies, indistinguishable from the bivalents formed by the small inversion-deficiency chromosome described in the previous section (Figs. 14, 15, 23, and 31). Sometimes inter-chromosome pairing had resulted in bivalents of double the size and with a median centromere (Fig. 24). Although the individual chromomeres could not be seen, and, consequently, a detailed comparison of the two arms of the large accessory chromosome was not possible, there can hardly be any doubt that the large accessory chromosome is an iso-chromosome, and that it has

originated through misdivision in the centromere of the inversion-deficiency chromosome. As described earlier, the centromere in this chromosome is terminal, no evidence for a second arm being found. It is, however, not very likely that any of the original breaks, which gave rise to the small type, were in the centromere itself. If so, one should have expected at least some new plants with large accessory chromosomes to have appeared in the crosses involving this small accessory chromosome. If this supposition be correct, the frequency of misdivision is also relatively low, although it may possibly vary from one plant to another.

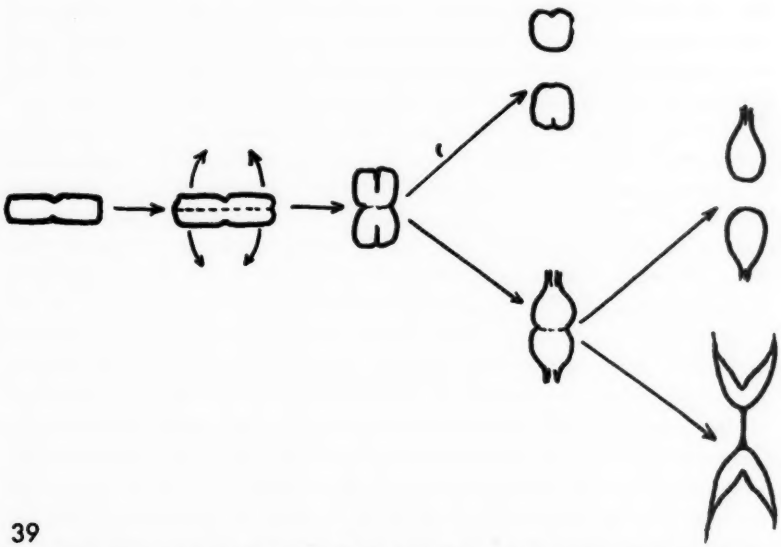
At diakinesis, in two plants with $2n=14+1$ st.$+1$ l. acc., the two types of accessory chromosomes were associated in about 45 per cent of the pollen mother cells, the corresponding figure at metaphase I being 10—15 per cent. In the plant with $2n=14+2$ st.$+2$ l. acc., which took part in the cross in 1954, the standard accessory chromosomes were nearly always associated at diakinesis, whereas 50 per cent of the large ones were present as univalents. Associations between standard and large accessory chromosomes were seen in only a small portion of the cells studied. At metaphase I, 85 per cent of the standard accessory chromosomes still formed a bivalent, corresponding to only 20 per cent for the large ones. Finally, in a plant with only two large accessory chromosomes, the percentages of associations at diakinesis and metaphase I were 59 and 2—3, respectively. This indicates that in the large accessory chromosome, inter-arm pairing is much more common than inter-chromosome pairing. Only when lacking a partner of their own kind do large and standard accessories associate to any extent. Similar to many of the associations between large accessory chromosomes seen at diakinesis, it is also probable that most of the configurations of standard and large accessory chromosomes are not the result of pachytene pairing and chiasma formation, but are instead due to an unspecific sticking together. The high percentage of univalent large accessories at metaphase I should, at later stages, result in an irregular transmission of this chromosome. The cytological studies as well as the results of the crosses have demonstrated that this is also the case. The positions of the univalents at metaphase I in the plant with $2n=14+$ $+2$ l. acc. are given in Scheme 1.

In 85 per cent of the I anaphases in the above-mentioned plant, one or two univalents were lagging between the poles. At telophase I, however, practically all the laggards had divided and were included in the daughter nuclei. Thus, the elimination at this stage is probably very

				Total
34	46	13	26	119

Scheme 1. The position of univalent large accessory chromosomes in a plant with $2n=14+2$ l. acc.

slight. In Figs. 26 and 35—38 the appearance of dividing univalents at anaphase I, large as well as standard, can be studied. As seen especially well in the large accessory chromosomes in Figs. 36 and 38, the chromatids move with their ends directed towards the poles. The same tendency has been observed in the standard type. This was already indicated by the figures in my earlier paper on the cytology of the standard



39

Fig. 39. Interpretation of the mode of division of univalent accessory chromosomes in *Festuca pratensis*. The different alternatives indicated at later stages of division are explained in the text.

accessory chromosome (BOSEMARK, 1954 a). At this stage of the investigation, however, the cytology of the large acccessory chromosome had not yet been studied, and the smallness of the standard type made a correct interpretation difficult. Similar to several cases of poleward movement of chromosome ends described in the literature, this phenomenon in *Festuca pratensis* is considered to depend on some kind of neo-centric activity of the chromosome ends. The whole subject will be considered at greater detail in the discussion. At anaphase II—telophase II in the plant with two large accessory chromosomes a fairly high percentage of laggards was observed. This resulted in a variable amount of tetrad micronuclei. From studies of very young pollen grains soon after the separation of the tetrad nuclei, it seems, however, as if a good deal of the micronuclei present at the early tetrad stage, have then been included in the main nucleus. In spite of this, the elimination of the large accessory chromosome is obviously sometimes very marked (Table 6 C).

The non-disjunction of the large accessory chromosome at the first pollen mitosis has also been cytologically verified. The appearance of large and standard accessory chromosomes at this stage is seen in Fig. 28. As in the standard type, the chromatids are held together on both sides of the centromere. As these segments in the standard type are probably both duplicated in the large one, it is possible that there are two points of attachment in each arm in the large accessory chromosome. If so, this might influence its ability to undergo non-disjunction. Contrary to the standard type, the chromatids of the large accessory chromosome are fairly often both included in the vegetative nucleus at the first pollen mitosis. The reason for this deviation is not known, but part of the loss of large accessory chromosomes in the progeny plants can probably be traced back to such non-disjunction directed to the vegetative nucleus instead of to the generative.

V. DISCUSSION

The process of directed non-disjunction of the standard accessory chromosome at the first pollen mitosis has been described in an earlier paper (BOSEMARK, 1954 a). Here, it was suggested that a specialized segment, heterochromatic or not, together with a modified centromere region, is responsible for the non-disjunction. The loss of this ability in the small accessory chromosome from plant 926 should then depend on the loss of this specialized segment, or on a defect in the centromere,

or on a combination of these events. In rye, the two iso-fragments, which have originated through misdivision of a standard fragment, differ with respect to their ability to undergo non-disjunction. The large iso-fragment, of which both the arms are identical with the long arm of the standard fragment (MÜNTZING and LIMA-DE-FARIA, 1949) has the same ability to undergo non-disjunction as the standard type, whereas the small iso-fragment, which is very likely a duplication of the short arm of the standard fragment, has entirely lost this ability. Similarly, a deficient standard fragment, which has been demonstrated to lack the terminal half of the long arm (MÜNTZING and LIMA-DE-FARIA, 1952), also proved to be unable to undergo non-disjunction. As the deficient accessory chromosome in rye has an intact centromere, these results clearly indicate that a segment necessary for the non-disjunction is situated in the distal half of the long arm, which is missing in the small iso-chromosome as well.

Similar to the results in rye, it is considered more likely that the loss of the right arm, in the small accessory chromosome from plant 926, is responsible for the loss of the non-disjunction, rather than that a change exclusively in the centromere should have led to this situation. The observations by ROMAN (1950) that the heterochromatic segment in the B-chromosome in maize is of importance for the non-disjunction, led MÜNTZING and LIMA-DE-FARIA (1952) to conclude that the distal heterochromatic knob in the long arm of the standard fragment in rye might actually be the seat of this activity. As most of the right arm of the standard accessory chromosome in *Festuca pratensis* consists of a large heterochromatic segment, it is thus likely that, also in this species, the seat of the activity is in such a segment.

The small accessory chromosome from plant 2311, which is, very likely, morphologically of the same type as that from plant 926, still has its non-disjunction mechanism unaffected when present together with standard accessory chromosomes in the pollen grain. When it is present alone in the pollen grain, however, this ability seems to be lost. This indicates that loss of the specialized segment in an individual accessory chromosome can be compensated for by the presence, in the same nucleus, of such segments in intact standard accessory chromosomes. The positive influence of the inversion-deficiency chromosome from plant 373 on the mechanism of non-disjunction of the standard type, as well as the corresponding effect of the large accessory chromosome, also correspond well with this conclusion. As shown in my earlier report (BOSEMARK, 1954 b), there is a marked variation between plants

with respect to the effectiveness of the non-disjunction mechanism. This variation is probably the result of a combined action of a number of factors, of which the genotype of the host plant was considered to be of special importance. The results now reported do not contradict such an opinion. Even if it is likely that there are inherent differences between different standard accessory chromosomes with respect to their ability to undergo non-disjunction, the genotype of the host plant may very well be the decisive factor in many cases. If the mechanism of non-disjunction is considered to be controlled by genes in the accessory chromosomes themselves, as suggested by ÖSTERGREN (1947), the products of these genes might be sufficient for a full manifestation of the non-disjunction mechanism in the pollen grains in some plants and under some conditions. In other plants, with other genotypes and under other environmental conditions, this might not be the case. Under such circumstances the addition of other accessory chromosomes, bringing about an increase in the necessary gene products, might result in a corresponding increase in the frequency of accessory chromosomes undergoing non-disjunction. Thus, the phenomenon now described should not be limited to an influence of standard or large accessory chromosomes on the smaller types. Owing to the irrregular meiotic pairing in plants with many accessory chromosomes, and to the high variability in all factors of probable importance for the non-disjunction, the detection of such an influence is, however, practically impossible in plants having large numbers of accessory chromosomes of only one morphologically distinct type.

If the above-mentioned hypothesis be correct, it is possible that also the small accessory chromosome from plant 926 should be capable of undergoing non-disjunction, if brought together with accessory chromosomes of the standard or large type. This then would indicate, as do also the data from plant 2311, that there is a second specialized segment or region, not situated in the right arm, which is also necessary for the manifestation of the non-disjunction, although alone it is not capable of bringing about the process. This region might very well be a modified centromere, an idea which is in conformity with the findings of ROMAN (1950) that in the B-chromosome in maize, the centromere as well as the heterochromatic segment in the long arm have significance for the non-disjunction. A process, such as the one outlined here will make, however, a strict delimitation of the number and positions of the segments involved a rather tedious work.

The origin of the small accessory chromosome in plant 373 through

an inversion in combination with a deficiency, explains the origin of the large accessory chromosome as well. As postulated earlier (BOSEMARK, 1954 a), the origin of this type from the standard is the result of a transfer of the centromere to a terminal position, followed by misdivision in the centromere and the production of an iso-chromosome. Although the origin of this iso-chromosome in *Festuca* is more complicated than that of the large iso-fragment in rye (MÜNTZING, 1944; MÜNTZING and LIMA-DE-FARIA, 1949), the behaviour at meiosis is similar in many respects (MÜNTZING, 1951). Thus, the degree of bivalent formation is in both cases lower than in the standard types, the decrease in inter-chromosome pairing being caused by the frequent inter-arm pairing. In both species this results in a high frequency of univalents at metaphase I. In rye, large iso-fragments and standard fragments from the variety »Östgöta Gråråg» pair at random when present together, with the production of hetero-bivalents as a result. Such pairing is much less frequent in *Festuca pratensis*, probably mainly due to the inversion in the arms of the iso-chromosome.

The behaviour of univalent accessory chromosomes at anaphase I probably constitutes a new case of neo-centric activity of chromosome ends. As mentioned already earlier, this activity is not confined to the large accessory chromosomes, but is very likely a common property of all types of accessory chromosomes in *Fectuca pratensis*. This was also pointed out to the author by Dr. D. L. HAYMAN. On the basis of his studies of two *Phalaris* hybrids (HAYMAN, 1955), and the figures in the paper of the cytology of the standard type of accessory chromosomes in *Festuca pratensis*, he suggested that the difficulties of univalent accessory chromosomes in dividing properly at anaphase I in some plants, and the drawn-out chromosome ends, might be due to some kind of active mobility of these ends. That this phenomenon is not always easily observed probably depends on factors not directly involved in the activity. Thus, the phenomenon is not clearly observable if the division of the lagging univalent takes place easily and soon after the separation of the bivalents. It is, however, likely that the chromatids of standard as well as large univalent accessory chromosomes are generally bent backwards already at a very early stage of division. If the division takes place without delay, the individual chromatids are situated so close to each other that it is generally impossible to note that even these chromatids, as a matter of fact, largely move with their ends directed towards the poles. If, for one reason or other, the separation is delayed, the ends are attenuated and the chromatids sometimes take the appear-

ance of an inverted V. This is illustrated by a schematic drawing (Fig. 39). A delay in the separation of the two halves of a univalent accessory chromosome can probably depend not only on the particular univalent itself, but also on the physiology of the host plant, as well as the number of univalents present. Evidence for such influence has earlier been obtained in *Festuca pratensis* (BOSEMARK, 1954 a and b). The reason for the poleward movement of the ends of dividing univalent accessory chromosomes in *Festuca pratensis* is not known. WALTERS (1952 a and b), in her works on atypical chromosome movement in *Bromus* hybrids, discusses several possible explanations. She arrives at the conclusion that some kind of neo-centromeres, the origin and nature of which are still obscure, are responsible for the poleward movement of the chromosome ends. The same explanation is given by HAYMAN (1955). In maize (RHOADES and VILKOMERSON, 1942; RHOADES, 1952), the so-called abnormal chromosome 10, which has a heterochromatic knob formation not present in the normal one, is capable of inducing neo-centric activity in normal chromosomes, especially in knob-bearing arms. In *Elymus Wiegandii,* one chromosome pair has an extra piece of deeply staining chromatin, not present in closely related species. The neo-centric activity observed in this species by VILKOMERSON (1950) has been associated with this extra chromatin. Also the T-ends in rye are found in arms probably possessing knobs (PRAKKEN and MÜNTZING, 1942; ÖSTERGREN and PRAKKEN, 1946; LIMA-DE-FARIA, 1952). As the accessory chromosomes in *Festuca pratensis* are largely heterochromatic, it is likely that also in this case heterochromatin is of significance for the manifestation of the neo-centromeres, and that its products in some way stimulate latent centromeric properties of the chromosome ends. The observation in maize that the presence of a normal centromere in the abnormal chromosome 10 is essential for the neo-centric activity, has not been possible to test in the present material.

SUMMARY

(1) Four derivatives of the standard accessory chromosome in *Festuca pratensis* were studied with respect to their cytology and inheritance. Three of the derivatives were smaller than the standard type, one was larger.

(2) Two of the small accessories had both lost the entire right arm, which consists mainly of a large heterochromatic segment. One of these small accessory chromosomes, which was present alone in the mother

plant, had completely lost its ability to undergo non-disjunction at the first pollen mitosis. The other one, which was present together with two standard accessory chromosomes in the mother plant, still had its non-disjunction mechanism unaffected when together with standard accessory chromosomes in the pollen grains, whereas when alone, this ability seemed to be lost.

(3) The third small accessory chromosome is only slightly shorter than the standard type. Its origin from the standard type involves a deficiency of the end of the left arm and an inversion transferring the centromere from a median to a terminal position. Its ability to undergo non-disjunction is not markedly changed. Good indications have been obtained of a positive influence of this small accessory chromosome on the non-disjunction mechanism of the standard type. There are tendencies also to a similar influence of the standard on the small type.

(4) The large accessory chromosome has originated from the deficiency-inversion chromosome, described in the previous paragraph, through misdivision in the centromere with the formation of an isochromosome as result. In crosses involving plants with 2 st.+2 l. acc., a significant positive influence of the large accessory chromosomes on the ability of non-disjunction of the standard type was found. Thus, in pollen grains having both large and standard accessory chromosomes, the effectiveness of the non-disjunction mechanism of the standard type was about double that of pollen grains with only standard accessory chromosomes. This effect of the large and small accessory chromosomes on the standard type, as well as the influence of the standard type on the small accessory chromosome earlier described, is explained on the basis of compensation for shortage or lack of gene products necessary for a full manifestation of the non-disjunction.

(5) At anaphase I dividing univalent large accessory chromosomes can often be seen to move their ends towards the poles. The same is very likely also true of the standard accessory chromosomes, although here the phenomenon is less easily observed due to the smallness of these chromosomes. It is suggested that the phenomenon constitutes a new case of neo-centric activity of chromosome ends, and that this activity is in some way connected with the presence of large heterochromatic segments in the accessory chromosomes.

Literature cited

BONNIER, G. and TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.

BOSEMARK, N. O. 1954 a. On accessory chromosomes in *Festuca pratensis*. I. Cytological investigations. — Hereditas XL: 346—376.

— 1954 b. On accessory chromosomes in *Festuca pratensis*. II. Inheritance of the standard type of accessory chromosome. — Hereditas XL: 425—437.

HAYMAN, D. L. 1955. Centromeric behaviour of the univalents in two *Phalaris* hybrids. — Aust. J. of Biol. Sci. 8(2): 241—252.

LIMA-DE-FARIA, A. 1949. The structure of the centromere of the chromosomes of rye. — Hereditas XXXV: 77—85.

— 1952. Chromomere analysis of the chromosome complement of rye. — Chromosoma 5: 1—68.

— 1955. Structure and behaviour of a chromosome derivative with a deleted kinetochore. — Chromosoma 7: 51—77.

MCCLINTOCK, B. 1933. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. — Z. f. Zellforsch. 19: 191—237.

MÜNTZING, A. 1944. Cytological studies of extra fragment chromosomes in rye. I. Iso-fragments produced by misdivision. — Hereditas XXX: 231—248.

— 1951. The meiotic pairing of iso-chromosomes in rye. — Portug. Acta Biol. (A), R. B. GOLDSCHMIDT Volumen: 831—860.

MÜNTZING, A. and LIMA-DE-FARIA, A. 1949. Pachytene analysis of standard fragments and large iso-fragments in rye. — Hereditas XXXV: 253—268.

— and — 1952. Pachytene analysis of a deficient accessory chromosome in rye. — Hereditas XXXVIII: 1—10.

PRAKKEN, R. and MÜNTZING, A. 1942. A meiotic peculiarity in rye, simulating a terminal centromere. — Hereditas XXVIII: 441—482.

RHOADES, M. 1952. Preferential segregation in maize. — Ch. 4 in »Heterosis» (Ed. J. W. GOWEN). — Iowa State College Press, Ames.

RHOADES, M. and VILKOMERSON, H. 1942. On the anaphase movement of chromosomes. — Proc. Nat. Acad. Sci. 28: 433—436.

ROMAN, H. 1950. Factors affecting mitotic non-disjunction in maize. — Abstract 1949 Meeting of the Genetics Society of America. — Genetics 35: 132—133.

WALTERS, M. S. 1952 a. Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid *Bromus marginatus*×*B. pseudolaevipes*. — Genetics 37: 8—25.

— 1952 b. Atypical chromosome movement in meiotic anaphase of *Bromus pitensis* ×*B. marginatus*. — Amer. J. Bot. 39: 619—625.

VILKOMERSON, H. 1950. The unusual meiotic behaviour in *Elymus Wiegandii*. — Exp. Cell Res. 1: 534—542.

ÖSTERGREN, G. 1947. Heterochromatic B-chromosomes in *Anthoxanthum*. — Hereditas XXXIII: 261—296.

ÖSTERGREN, G. and PRAKKEN, R. 1946. Behaviour on the spindle of the actively mobile chromosome ends of rye. — Hereditas XXXII: 471—494.

BRIEF REPORTS

OVE L. HALL: Further experiments in embryo transplantation.

In analysing the genetic background to the crossability of wheat and rye, several investigators have used as their object of study a Chinese spring wheat which BIFFEN (BIFFEN and ENGLEDOW, 1926) brought home from the province of Szechuan in China. It is distinguished by the quality of offering practically no crossing barrier to rye (BACKHOUSE, 1916; LEIGHTY and SANDO, 1928; TAYLOR and QUISENBERRY, 1935; LEIN, 1943).

The above mentioned variety of Chinese wheat also offers interesting material for the investigation of the nature of the crossing barrier between wheat and rye, which is now in progress at the Institute of Genetics at Lund. Experiments on the effect of embryo transplantations on the crossing barrier (HALL, 1954) showed that wheat plants which were able to utilize rye endosperm during their early development could be more easily crossed with rye than wheat plants which had grown on homologous endosperm. In order to ascertain if endosperm from the easily crossable Chinese wheat has the same effect as rye endosperm on a variety of wheat that is difficult to cross with rye, transplantation experiments were made in 1955 as described below.

The experiment was started on the 19th of April, when 500 embryos of Ella wheat were transplanted on to endosperm of the Chinese wheat. At the same time, for purposes of comparison, 500 Ella embryos were transplanted on to Ella endosperm, and a further 500 Ella embryos on to the endosperm of Od spring rye. These three groups may be denoted E/Ch, E/E and E/Od. The transplantation technique and the procedure in general are described in my paper of 1954. The grafted kernels were put in sterilized soil in small pots, which were then placed in a greenhouse. On the 25th of May the small plants, which were then 1—1 $^1/_2$ dms. high, were moved out on to the field. The three groups grew there at a normal speed, and no difference in their vigour was observable. During the period 4th to 8th July 2.514, 2.177 and 5.662 flowers were emasculated within the three groups E/Ch, E/E and E/Od. The emasculated ears were isolated in pergamyn bags and after 4—5 days the flowers were pollinated with Od-rye pollen.

The result of the pollination was that the E/E group, *i. e.*, the control group, developed 9, the E/Ch group 33 and the E/Od group 92 hybrid kernels corresponding to a rate of seed setting of 0,4, 1,3 and 1,6 per cent. The three groups had been treated in exactly the same way, thus allowing direct comparisons. The significance of the difference in the amount of kernels obtained from the groups E/E, E/Od and E/Ch can be verified according to the χ^2 method below.

	Number of unsuccessful crosses	Number of successful crosses	n
E/E	2168	9	2177
E/Od	5570	92	5662
E/Ch	2481	33	2514
Total	10219	134	10353

The χ^2 value for heterogeneity is 18,3, which for two degrees of freedom corresponds to a P-value less than 0,001. For one degree of freedom, corresponding to a comparison between the control group and the two others, the χ^2 value is 16,7 and P is less than 0,001. No significant difference exists between the groups E/Od and E/Ch. *From the above it is clear that the Chinese wheat endosperm, similarly to the rye endosperm, influenced the Ella wheat by reducing the effectiveness of the crossing barrier between Ella wheat and rye.*

The extremely hot weather at the time of emasculation and pollination was probably the cause of the low degree of seed setting obtained. In an experiment with an E/Od group a month earlier, when the weather was considerably cooler, 1.911 flowers were emasculated and pollinated with rye, and from these 172 hybrid kernels were obtained. This corresponds to 9 per cent of seed setting. In 1953 the percentage of successful crosses in the E/E group was 2,6 per cent and for the E/Od group 13,8 per cent. At that time also the day temperature was lower during the pollination period. The results of the crosses in 1955 between Chinese wheat and Od, which were carried out in July, also indicate that the conditions at that time were not favourable for hybridization experiments. The rate of successful crosses was only 41 per cent, a very low number for that particular cross combination. BACKHOUSE obtained 80,0 per cent of seed setting, LEIGHTY and SANDO 90,5, TAYLOR and QUISENBERRY 69,0, and LEIN 66,6 as a mean value for four years.

Transplantation experiments with Ella and Chinese wheat will be repeated this year, and the comparative biochemical studies of these two wheat varieties which are now being carried out will be continued.

Institute of Genetics, Lund, Sweden.

Literature cited

BACKHOUSE, O. W. 1916. Note on inheritance of crossability. — Journ. Gen. 6: 91—94.

BIFFEN, R. H. and ENGLEDOW, F. L. 1926. Wheat-breeding investigations at the Plant Breeding Institute. — Cambridge, Min. of Agr. and Fish. Res. Monogr. No. 4: 1—114. London.

HALL, O. L. 1954. Hybridization of wheat and rye after embryo transplantation. — Hereditas XL: 453—458.

LEIGHTY, C. E. and SANDO, W. J. 1928. Natural and artificial hybrids of a Chinese wheat and rye. — Journ. Hered. 19: 23—27.

LEIN, A. 1943. Die genetische Grundlage der Kreuzbarkeit zwischen Weizen und Roggen. — Zeitschr. f. ind. Abst. und Vererb. 81: 28—61.

TAYLOR, J. W. and QUISENBERRY, K. S. 1935. Inheritance of rye crossability in wheat hybrids. — Journ. Amer. Soc. Agron. 27: 149—153.

TILL MEDARBETARNA

Manuskript — helst *maskinskrivna* — torde insändas till redaktionen (Genetiska Institutionen, Lund) i fullt tryckfärdigt skick. De böra vara *noga genomsedda* för undvikande av ändringar mot manuskriptet. Obs. kommateringen! Korrektionskostnaderna betalas av författaren. Korrektur ställes till redaktionen. Direkt förbindelse mellan författaren och tryckeriet tillåtes icke.

Personnamn sättas med KAPITÄLER. De markeras i manuskriptet med en våglinje. Latinska namn på växter och djur samt ord och satser av särskild vikt *kursiveras* (enkel understrykning).

Figurer numreras med arabiska siffror. Figurförklaring (på avhandlingens språk) torde insändas *samtidigt* med illustrationsmaterialet.

Planscher numreras med romerska siffror och de i dem ingående bilderna med arabiska.

Tabeller åsättas arabiska siffror och förses med kort rubrik.

Citerade arbeten samlas i en litteraturförteckning. I texten hänvisas till denna genom angivande av författare och årtal. Har en författare utgivit flera publikationer under samma år, tilläggas efter årtalet små bokstäver (a, b, c, etc.). Samma beteckningssätt användes i litteraturlistan, vilken uppställes i alfabetisk ordning efter författarna och under dessa i kronologisk följd. Inga litteraturhänvisningar få göras genom fotnoter. Överhuvudtaget så få noter som möjligt!

Avhandlingarna skola vara skrivna på engelska, tyska eller franska. Det är önskvärt, att uppsatser på tyska och franska åtföljas av en resumé på engelska. Översättningar, som ombesörjas av redaktionen, bekostas av författaren.

Åt varje författare lämnas 100 fria separat. Avhandlingar på ett ark och däröver förses gratis med särskilt omslag. Till ett pris av 15 kr. pr 100 st. lämnas, om så önskas, omslag till mindre uppsatser. Större antal särtryck fås till självkostnadspris. *Anteckning om eventuella extraseparat göres å första korrekturet.*

Hereditas is open to Scandinavian writers only (and to foreigners working at Scandinavian institutes).

INNEHÅLL

TJIO, JOE HIN and LEVAN, ALBERT, The chromosome number of man 1

NORDENSKIÖLD, HEDDA, Cyto-taxonomical studies in the genus *Luzula* II. Hybridization experiments in the *campestris-multiflora* complex 7

NYBOM, N., GUSTAFSSON, Å., GRANHALL, I. and EHRENBERG, L., The genetic effects of chronic gamma irradiation i barley 74

LIMA-DE-FARIA, A., The role of the kinetochore in chromosome organization .. 85

OKSALA, TARVO, The mitotic mechanism of two mouse ascites tumours 161

BOSEMARK, NILS OLOF, On accessory chromosomes in *Festuca pratensis* III. Frequency and geographical distribution of plants with accessory chromosomes 189

NYBOM, NILS, On the differential action of mutagenic agents 211

TJIO, JOE HIN and LEVAN, ALBERT, Comparative idiogram analysis of the rat and the Yoshida rat sarcoma 218

BOSEMARK, NILS OLOF, On accessory chromosomes in *Festuca pratensis* IV. Cytology and inheritance of small and large accessory chromosomes 235

HALL, OVE L., Further experiments in embryo transplantation 261

HEREDITAS

GENERAL INDEX

INDEX OF PAPERS. SPECIES AND SUBJECT INDEX

VOLS. 1–40 (1920–1954)

Recently published

Price 15 Swedish Kronor

Postage free

Orders to be sent to

Dr. PETER BERNSTRÖM

Hilleshög Sugar Beet Breeding Institute

Landskrona, Sweden

Utgivet den 15 maj 1956.